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## Green Tea Polyphenols as Potential Food Additives

Danxia Shi  
*Montclair State University*

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## ABSTRACT

As time has progressed, companies have increasingly produced foods for longer term storage. This has led to an increased use of food preservatives in processed foods. People have now come to expect many foods to have a significantly longer shelf life, and this is a matter of government concern for public health due to foodborne contaminations. Foodborne illnesses have become a common problem caused by bacteria (i.e. *Escherichia coli*). Finding an effective natural preservative source might be better received by the public and alleviate some health concerns over chemical sources.

The most important active ingredient found in green tea is camellia *sinensis*. Epigallo-cathechin-3-gallate (EGCG) is the primary active polyphenol in camellia *sinensis*. Palmitoyl-epigallocatechin-3-gallate (P-EGCG), and epigallocatechin-3-gallate-stearate (EGCG-S) are two modified tea polyphenols which were studied in the current experiments. Green tea extracts contain antioxidant, antimicrobial, antimutagenic, and anticarcinogenic properties.

In this study, the stability of EGCG, P-EGCG and EGCG-S were determined by the incubation rates of *Escherichia coli*; *Lactobacillus bacillus*; and *Streptococcus thermophiles*. The green tea polyphenols' antimicrobial properties were evaluated at different temperature and pH conditions. The temperatures used in the current studies were: 27 °C (room temperature); 37°C, 55°C, and 68°C (three pasteurized conditions: 68°C water bath 24h, 30min 68°C water bath before 24h 27°C treating, 30min 68°C water bath after 24h 27°C treating). The nutrient broth (pH=7.0) and clementine orange juice (pH=3.03) were used to determine the stability of EGCG, P-EGCG and EGCG-S. The stable effect of EGCG, P-EGCG and EGCG-S were analyzed through Colony Forming Units (CFU). The next objective of this study was to determine the antioxidant effect of EGCG, P-EGCG and EGCG-S with clementine orange juice through the UV-spectrometer. According to the data analysis, it suggests that P-EGCG is the most effective tea polyphenol in reducing bacteria growth and maintaining antioxidant properties of clementine orange juice during storage. Therefore, P-EGCG could be considered as having the best potential among the tested polyphenols as a citrus juice preservative.

MONTCLAIR STATE UNIVERSITY  
GREEN TEA POLYPHENOLS AS POTENTIAL FOOD ADDITIVES

by

Danxia Shi

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

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Thesis Sponsor:  
Dr. Charles Feldman



Committee Member:  
Dr. Shahla Wunderlich



Committee Member:  
Dr. Lee Lee

**GREEN TEA POLYPHENOLS AS POTENTIAL FOOD**

**ADDITIVES**

**A THESIS**

**Submitted in partial fulfillment of the requirements**

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**Montclair State University**

**Montclair, NJ**

**2017**

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## **INTRODUCTION FOR STUDY #1 AND STUDY #2**

Longer food storage times have become increasingly important and necessary to reduce or eliminate foodborne contaminants. About 1000 years ago, in order to survive in the winter, people used ice to store food (Chassy, 2007). Currently, the problem of the best method of how to keep fresher food longer is a matter of ongoing research. Modern food production frequently includes chemical food additives. However, chemical food preservatives increases concerns of consumers (Cleveland, Montville, Nes, & Chikindas, 2001). Many people are interested in natural food preservatives over chemical-based preservatives. Researchers are looking into natural alternatives to address these concerns.

### **1. Foodborne bacteria**

Foodborne illness outbreaks occur frequently worldwide. Eating or drinking contaminated food or beverage often causes foodborne illnesses. Usually these contaminated foods contain high level of toxins (chemicals) and/or of pathogens such as bacteria, viruses and parasites (Gabida et al., 2015). Foodborne diseases and foodborne pathogens not only cause health problems, but also negatively influence food safety. Scientists and manufacturers have used many techniques to avoid the spreading of foodborne illnesses, but results vary and research continues to develop methods of rapid detection of food borne pathogens (Mandal, Biswas, Choi, & Pal, 2011). Most pathogens such as Salmonella will be found in animal sources. The pathogens can also spread virus' to people and animals, and influence their health (Callaway, Edrington, Harvey, Anderson, & Nisbet, 2012). Most contaminated foods were attributable to unsafe production and processing (Gabida et al., 2015). In addition, unprofessional and unclean food serving



habits are one of the important sources of transmitting foodborne illness to customers (Mitchell, Fraser, & Bearon, 2007).

*Escherichia coli*, especially *Enterohaemorrhagic Escherichia coli*, is a pathogen spread worldwide, and it can cause high numbers of infections. In addition, *Enterohaemorrhagic Escherichia coli* is one of the zoonotic pathogens which cannot only cause diarrhea, but can also rarely cause thrombocytopenia in children and adults (Ambrožová & Marejková, 2011). People who eat undercooked meat products, and intestines from animals may be infected by *Enterohaemorrhagic Escherichia coli*. These organisms from animal products could produce a number of virulence factors that can cause intestinal illnesses like bloody diarrhea and hemolytic urinemic syndrome (Bettelheim, 1996).

According to all the information above, in order to prevent foodborne illness, people should be concerned with food manufacture and the manufacturing process, but also focus on how to decrease rates of pathogenic infections.

## **2. Food additives**

There has been a long developmental history of food additives. As the technology develops, food preservation is becoming more and more complex. By the middle of the 20th century, processed food has become an important part of people's lives. For the modern food industry, chemical food additives become important part of food preservation. Today food manufacturers use more than 2500 additives which benefits both the manufacturer and the consumer economically, as mass production reduces costs. However, food additives can have an adverse health effect. Some of the chemical food additives are suspect due to their supposed or potential toxicity (Nychas, 1995). Many of these additives

have been banned, but only a few of them are banned at a global level (Shin, Artigas, Hobbie, & Lee, 2013). Although many additives are safe, some are not; natural food preservatives are generally considered to be the safest option for keeping foods fresh (Singh, Saengerlaub, Wani, & Langowski, 2012).

Scientists found that some chemical additives have positive impacts on processed food flavor and storage time (preserving juice, processing vegetables) (Alam, Hoque, Morshed, Akter, & Sharmin, 2013; G. Kaur & Aggarwal, 2015; Olotu et al., 2015), but some have turned out to have negative effects on human health, such as triphenylmethane dyes, amaranth, cyclamates and possibly saccharin (suspected carcinogen, not fully established) (Fairweather & Swann, 1981; Mischek & Krapfenbauer-Cermak, 2012; Nakonieczna, Paszkowski, Wilczek, Szyplowska, & Skierucha, 2016). In addition, a high intake of benzoic acid could cause some adverse health effects, such as metabolic acidosis, hyperpnoea and convulsions (Mischek & Krapfenbauer-Cermak, 2012). Research showed that increasing intake of processed meats could increase the risk of cardiovascular disease (CVD) (Powles et al., 2013).

Some natural products, especially natural antioxidants, and antimicrobial agents, could be safe alternatives to synthetic compounds. For example, Polyphenols are natural antioxidants which are commonly extracted from fruits, vegetables, beverages (tea, wine, juices), plants, seaweeds, and some herbs. Polyphenols have high antioxidant and antimicrobial abilities when used in various fish and fish products (Maqsood, Benjakul, & Shahidi, 2013). Other natural food additives, such as tasmania lanceolate solvent extracts, eucalyptus essential oil, and tarragon (*Artemisia dracunculus*) essential oil (TEO), all have antioxidant, and antibacterial properties to fight bacteria and fungi growth, and are not

harmful to human health. In addition, TEO can prevent food yeast (Hart et al., 2014; B. Ifesan, Siripongvutikorn, Hutadilok-Towatana, & Voravuthikunchai, 2009; Pedjie, 2010; Sharafati-Chaleshtori et al., 2014; Tyagi, Gottardi, Malik, & Guerzoni, 2014). So, natural food additives may be safer alternatives.

### **3. EGCG, P-EGCG and EGCG-S**

Green tea is a healthy food resource, and green tea extract is a substance with perhaps a future as a food preservative. Green tea of different varieties has been grown in over 30 countries. People consume green tea primarily in China, Japan, a few countries in North Africa and the Middle East (Graham, 1992). Green tea is often considered as a potent medicine for keeping people healthy in China and Japan. People in these countries believe green tea has the power to help them prolong their lives (Balsaraf & Chole, 2015). Green tea is a 'non-fermented' tea, and it contains many catechins, which have strong antioxidants in vitro and in vivo (Cabrera, Artacho et al. 2006). Nowadays, people use green tea widely for diabetes, exercise enhancement, inflammatory bowel disease, skin disorders, hair loss, weight loss, and iron overload (Sinija & Mishra, 2008). Most of the health benefits of teas come from the antioxidant content in the *camellia sinensis* (du Toit, Volstedt et al. 2001). The ultimate antioxidative and bioactive ability in green tea is dependent on the bodies' absorption, distribution, metabolism, and excretion (ADME) properties of the catechins (Balsaraf & Chole, 2015). Scientists have shown that intake of green tea can effectively reduce the possibility breast cancers in animal subjects (Jankun, Selman et al. 1997). It is also known for its anti-carcinogen, anti-inflammatory, anti-bacterial, and anti-viral properties (Dai, Chen et al. 2008). Many of these properties are also effective in controlling weight loss, and arrests the exacerbation of pulpitis (Balsaraf & Chole, 2015).

Flavonoids are the major polyphenols in green tea. The four major flavonoids in green tea are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) (Siniya & Mishra, 2008). Also, epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC) and epicatechin-3-gallate (ECG) are all included in catechins (Cabrera, Artacho et al. 2006). These epicatechins can be modified to become non-epicatechins from their original formulation, such as galocatechin-gallate (GCG), catechin-gallate (CG), galocatechin (GC) and catechin (C) (Ananingsih, Sharma et al. 2013). EGCG is the most abundant and active catechin in all of these epicatechins (Lakenbrink, Lapczynski, Maiwald, & Engelhardt, 2000; H. Wang & Helliwell, 2000; R. Wang, Zhou, & Jiang, 2008). Even though, there are other polyphenols in green teas, such as gallic acid, quercetin, kaempferol, myricetin and their glycosides, the concentrations are lower than EGCG normally (Sakakibara, Honda et al. 2003).

Epigallocatechin-3-gallate (EGCG) can prevent carcinogenesis (Tachibana, Koga et al. 2004). Also, epigallocatechin gallate (EGCG) can be used to reduce the risk of CAD (Hirun & Roach, 2005). Moreover, epigallocatechin-3-gallate (EGCG) had a positive effect on inhibiting the growth of *Streptococcus mutans*, and *Staphylococcus aureus* (Cui et al., 2012; Sakanaka, Kim, Taniguchi, & Yamamoto, 1989). The antioxidant abilities of EGCG could be a natural food additive to help store fish longer and keep it fresher (Zhong & Shahidi, 2012). In addition, EGCG can modify a molecule for enhanced lipophilicity to improved cellular absorption in vivo (Zhong & Shahidi, 2012). EGCG is unstable at high temperatures and at alkaline pH. So, it may not work well as an ingredient in certain food products (Hirun & Roach, 2005). However, EGCG showed high stability in strawberry

sorbet, which means EGCG can inhibit bacteria in a higher acid pH and at lower a temperature condition (Hirun & Roach, 2005).

A modified EGCG polyphenol, palmitoyl-epigallocatechin-3-gallate (P-EGCG), showed that it can effectively inhibit herpes simplex virus 1 (HSV-1) infections in experiments (De Oliveira, Adams et al. 2013). Thus, P-EGCG is a green tea extract to be considered in a food science study. Epigallocatechin-3-gallate-sterate (EGCG-S) is another polyphenolic compound in green tea, which belongs to epigallocatechin gallate, was valued in the present study. The fundamental components of interest of this research are the polyphenols found in green tea, especially in *camellia sinensis*, which are the native evergreen shrubs in East Asia (Dai, Chen et al. 2008).

#### **4. Antioxidant evaluate (Vitamin C)**

There are many studies that have analyzed the effect of antioxidant properties of vitamin C (Bailey, Williams, Betts, Thompson, & Hurst, 2011; Jacob & Burri, 1996; Levine et al., 1996). Vitamin C could act as a nutrient marker in the current study to evaluate whether these green tea polyphenols could destroy vitamin C and antioxidant properties. The high availability of antioxidants in vitamin C assists people in resisting a variety of diseases, for example, it could decrease the likelihood of getting the cold (Jacob & Burri, 1996). Vitamin C could also serve as an antioxidant component in plasma, as well as the extracellular fluids surrounding the lungs, lens and retina to prevent free radicals from attaching to healthy cells. The protection of phagocytic cells by vitamin C involved in the defense against pathogenic invasions (Bendich, Machlin, Scandurra, Burton, & Wayner, 1986). In addition, research has found that moderate intake of vitamin C could protect people's pulmonary functions (Schwartz & Weiss, 1994). The researchers checked

the biochemical, clinical, and epidemiologic evidence to find if nonsmokers ingested of 90–100 mg vitamin C daily, they could effectively reduce the opportunity to get chronic diseases (Carr & Frei, 1999). The above studies have shown that vitamin C is an important component of the overall antioxidant protective mechanisms in cells and tissues.

Many vegetables and fruits are naturally rich with vitamin C, for example oranges, strawberries, and tomatoes (S. K. Lee & Kader, 2000). Scientists found that high hydrostatic pressure (500 MPa, 35 °C, 5 min) before pasteurizing helped juice extended its shelf life at temperatures under 5 degree conditions (Polydera, Stoforos, & Taoukis, 2003). Also, ascorbic acid degradation rates in orange juice were lower in high pressure processes (Polydera et al., 2003). In addition, the longer orange juice was stored, the more the vitamin C's potency decreased (H. Lee & Coates, 1999). Moreover, vitamin C in citrus drinks statistically had higher levels of antioxidants, but showed lower levels in apple and pineapple juice (Gardner, White, McPhail, & Duthie, 2000). Polyphenols and vitamin C are important contributors of antioxidant properties in fruits, and some fruits, which contain different genes, also had different antioxidant abilities (Du, Li, Ma, & Liang, 2009).

Different genotypes, climatic conditions, harvesting methods, and postharvest procedures, could all change vitamin C content (S. K. Lee & Kader, 2000). The TEAC (Trolox Equivalent Antioxidant Capacity), FRAP (Ferric Reducing Ability of Plasma) and ORAC (Oxygen Radical Absorbance Capacity) values for each plant's extract showed a similar and well-correlated with the total phenolic and vitamin C contents (Proteggente et al., 2002).

## 5. Thesis Design

This thesis is based on two research components. Section one in this thesis is a literature review which focuses on the background of chemical and natural food additives, and of foodborne bacteria. In addition, the reason that the detection of vitamin C levels in clementine orange juice was required. The definitions of green tea polyphenols were also discussed. Section two (Manuscript #1) presents the percentage of inhibition of *escherichia coli*, *lactobacillus bacillus* and *streptococcus thermophiles* with three tea polyphenols (EGCG, P-EGCG, EGSG-S) at different temperature conditions. The bacterial inhibition rate can determine whether the green tea polyphenols were stable in different treatment conditions. Section three (Manuscript #2) covers the detection of the stability of effective levels of the three tea polyphenols (EGCG, P-EGCG, EGCG-S) in clementine orange juice at pasteurization. As food preservatives, whether the green tea polyphenols could destroy the nutrient value of food is significant. This manuscript explains whether P-EGCG, and EGCG-S retain vitamin C and antioxidant properties in clementine orange juice using a UV- spectrometer. presents the percentage of inhibition of *escherichia coli*, *lactobacillus bacillus* and *streptococcus thermophiles* with three tea polyphenols (EGCG, P-EGCG, EGSG-S) at different temperature conditions. The bacterial inhibition rate can determine whether the green tea polyphenols were stable in different treatment conditions. Section four presents conclusions and recommendations, which results from the findings in manuscripts #1 and #2. The Appendices in the manuscripts provide further supporting information.

## **MANUSCRIPT #1**

# **The Study of Stability of Three Green Tea Polyphenols in Foodborne Bacteria and Beneficial Bacterium Condition**

Danxia Shi BS1\*, Charles Feldman PhD1, Chia-liang Huang BS1,  
Shahla Wunderlich PhD1, Lee Lee PhD2

<sup>1</sup>Montclair State University, Department of Nutrition and Food science; and <sup>2</sup>Department of Biology, NJ 07043, USA

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\*Author for correspondence. Tel: (862) 213-1573; E-mail: shid3@montclair.edu



## **ABSTRACT**

To consider the needs of natural food preservative, three tea ingredients were evaluated in this research. Epigallocatechin gallate (EGCG), palmitoyl-epigallocatechin gallate (P-EGCG), and epigallocatechin-3-gallate-stearate (EGCG-S) from green tea have the potential ability to reduce food related bacteria growth. The aim of the study is to examine the stability of EGCG, P-EGCG, and EGCG-S in the nutrient broth (pH 7.0), and fresh clementine orange juice (pH 3.03) at the entire temperature range (25°C - 68°C). EGCG became unstable at 55°C and inactivated at temperature 68°C. However, P-EGCG, and EGCG-S inhibited bacterial growth 100% at high temperature and in pasteurized clementine orange juice (acid juice environment). The stability of these three green tea extractions in nutrient broth and in fresh clementine orange juice were compared. The data showed that EGCG, P-EGCG, and EGCG-S inhibit more bacteria at clementine orange juice condition ( $p < 0.05$ ). In addition, the results showed that green tea polyphenols were the most important factor among this study to inhibit bacteria growth ( $p < 0.05$ ). P-EGCG had the best potential for reduction of bacterial growth in the study. P-EGCG could be considered to as the most stable green tea extraction to inhibit foodborne bacteria growth.

**Key words:** Green tea extractions; Pasteurized temperature; Low pH; Stable assessment; Clementine orange juice; stability comparison

## INTRODUCTION

As technology develops, food preservation is becoming more complex. Chemical food additives are very important in the modern food industry. For this reason, toxic chemical additives also can affect the safety of processed food. Thus some chemical food additives are suspect due to their potential or perceived toxicity (Heudorf, Merschundermann, & Angerer, 2007; Nychas, 1995). Manufacturers tend to develop chemical preservatives for their food products, while others adopt more natural alternatives for their products. (Holley & Patel, 2005; Nychas, 1995). Natural additives are widely used in numerous consumed items such as: cough syrup; toothpaste; dairy, fruit juice (Tyagi et al., 2014). Natural antioxidants, and antimicrobial agents, could be considered as safer alternatives to synthetic compounds (Hart et al., 2014; Maqsood et al., 2013). Food manufacturers often utilize these natural alternatives as preservatives, acidulates and stabilizers (B. O. Ifesan, Siripongvutikorn, & Voravuthikunchai, 2009; Pedjie, 2010).

Often, food preservatives can increase the shelf life of food products, and help food have a longer shelf-life. In addition, preservatives can help prevent vegetable and fruit spoilage. Furthermore, some preservatives can improve the nutritional content, and preserved color, taste, and nutritive values of food. Thus, whether food preservatives can extend shelf life in different temperature, and acid-base environments is a worthwhile problem to be explored.

In modern food industry, food safety is taken seriously. Contaminated food or beverages could cause foodborne illness. High levels of pathogens and toxins (chemicals) such as bacteria, viruses, and parasites are often present in contaminated food (Gabida, Gombe et al. 2015). *Escherichia coli* exists worldwide as a pathogen that can spread.

*Enterohaemorrhagic Escherichia coli* is one of the zoonotic pathogens which cause diarrhea, even more, the thrombocytopenia in children and adults (Ambrožová & Marejková, 2011). Normally, *E. coli* are found in undercooked meat products, and intestines from animals (Bettelheim, 1996). The FDA in US reported that *E. coli* can be killed at 155°F -165°F (68°C- 73°C) for 15 seconds (U.S. Food and Drug Administration, 2014). So, properly cooked foods are much safer than undercooked foods.

EGCG is the most abundant and active catechin among all of these epicatechins (Lakenbrink et al., 2000; H. Wang & Helliwell, 2000); palmitoyl-epigallocatechin gallate (P-EGCG), and Epigallocatechin-3-gallate-stearate (EGCG-S) are modified versions of the primary molecule EGCG. In stability studies of green tea catechins, the phytochemical product has been under investigation for decades. Green tea extract (GTE) as a source of tea catechins was stable in frozen and unfrozen environments (R. Wang & Zhou, 2004). In addition, the storage temperature does affect the stability and effectiveness of GTE (H. Wang & Helliwell, 2000; Widyaningrum, Fudholi, Sudarsono, & Setyowati, 2006). Studies have shown that EGCG is stable at low storage temperatures or around 2°C (Fangueiro et al., 2014; Widyaningrum et al., 2006). UPLC and Matlab programming showed that EGCG had higher stabilities than ECG above 44 °C (Xu, Wei, Ge, Zhu, & Li, 2015).

EGCG can work well in inhibiting bacteria growth, and can have health promoting effects in treating cancers and herpes patients (Xiong et al., 2017). Two studies demonstrate, EGCG does not tolerate higher temperatures. However, these findings may not be conclusive. Another look at tea polyphenol's potential for food safety may uncover potential effective applications (Friedman, Levin, Lee, & Kozukue, 2009; Li, Du, Jin, & Du, 2012). So, whether P-EGCG and EGCG-S have a higher temperature tolerance ability

than EGCG will be valuable to consider in the current study. Some research has demonstrated that EGCG could be more effectively in inhibit fungus in clinics (Guida et al., 2013; Mittal, Pate MSWylie, Tollefsbol, & Katiyar, 2004; Park et al., 2006). Adding EGCG and EGCG-S to processed foods to determine the antibacterial ability will be a very important role for future studies of EGCG-S.

Tea catechins are more stable in aqueous solutions with a pH value below 4, whereas tea catechins would be unstable in solutions with a pH value above 6. Studies have also shown that EGCG is inactive in a high acid environments (Ananingsih, Sharma, & Zhou, 2013; Z.-Y. Chen, Zhu, Wong, Zhang, & Chung, 1998). Thus, EGCG would be a less effective choice for treating higher acidic foods. The present study is to find whether P-EGCG and EGCG-S have high acid resistance.

## **MATERIALS AND METHODS**

The thermostability of EGCG, P-EGCG, and EGCG-S were determined by the CFU of *L. burglarious*, *S. thermophiles* and *E. coli* at four temperatures: 25°C (room temperature); 37°C; 55°C; and at 68°C (in long-term, low-temperature pasteurization). The pH stability of EGCG, P-EGCG, and EGCG-S were tested at the above temperature conditions using clementine orange juice, pH=3.03. The Biology Laboratory had three suitable bacteria that were obtained and used in this current study. *L. burglarious*, and *S. thermophiles* are two positive- gram bacteria that can survive in a higher acid condition. In addition, *S. thermophiles* can survive well at higher temperatures. *E. coli* are a large, diverse groups of bacteria, which commonly cause foodborne illnesses.

## 1. Bacterial Strains and Cultures

The following bacteria were used in this study: (1) *Escherichia coli*, provided by the Biology laboratory at Montclair State University; (2) *Lactobacillus burglariou*s, isolated from homemade yogurt in the laboratory; (3) *Streptococcus thermophiles*, isolated from same homemade yogurt (in laboratory). *E. coli*, *L. burglariou*s, or *S. thermophiles* were passaged weekly onto nutrition agar (Difco™) plates followed by an overnight incubation (24 h at 37°C) and then placed for one month in storage in a cold room at 4°C as stock. This process was repeated as needed to maintain a stable stock. For each experiment, the bacteria cultures were harvested after being incubated at 37°C overnight. Then the cultures were transferred to the nutrition broth (Difco™) to normalize the population of bacteria in OD600 of 0.1 units in both control and treatment settings. If contamination was present in the original stock, the bacteria were isolated.

## 2. Tea Reagents and Chemicals

Three tea extractions: epigallocatechin-3-gallate epigallocatechin gallate (EGCG), Polyphenols epigallocatechin (P-EGCG), and epigallocatechin-3-gallate-stearate (EGCG-S) —were evaluated in the experiment. For all experiments, polyphenol stock solutions were freshly prepared in ethanol. All tea compounds were prepared at the final concentration of 5mg/ml.

All of the other chemicals and reagents were purchased from Thermo Fisher Scientific Co. (Waltham, MA, USA).

## 3. Clementine Orange Juice Preparation

Clementines (*citrus reticulata*) were procured for experimentation on January 18, February 20, and March 3, 2017. These particular "Darling Clementines," grown in

California and exclusively trademarked and marketed by LGS Specialty Sales, New Rochelle, NY, were purchased at Shoprite in Bloomfield, NJ. The clementine oranges juice was obtained, after suitable washing and a 10-minute ultraviolet radiation sterilization processing in the biology lab. A fresh clementine orange was squeezed to produce juice in a flask. The pH of the sample juice was then tested.

#### 4. Stability of Tea Extractions in Different Temperature

CFU assay was used for determining the viability of bacterial colonies in the stability study. The prepared *L. burglarious* (LB), *S. thermophiles* (ST) and *E. coli* (EC) were used to assess the themostability of EGCG, P-EGCG, and EGCG-S. An OD600 of 0.1 unit bacteria (LB, ST, EC) broth were used to make the 4X 90ul treatment base solutions separately. 10ul of tea stock (5mg/ml of EGCG, P-EGCG and EGCG-S) were put into 90ul of bacteria (LB, ST, EC) solutions as experimental groups. 10ul of nutrient broth was added into bacteria (LB, ST, EC) agents as a control. Control and experimental groups were kept in the temperatures of experiments 1 & 2 for 24 hours. After a one day treatment, a serial dilution was performed with DI water for both control and experimental groups. Plated samples were placed on nutrient agar plates in a countable range. Then, plates were put into a 37 C incubator overnight. The colonies were counted and % of inhibition was determined.

The equation of percentage of inhibition was:

$$\% \text{ of Inhibition} = \left( \frac{\text{CFU}_{\text{Control}} - \text{CFU}_{\text{Treated}}}{\text{CFU}_{\text{Control}}} \right) \times 100$$

#### 5. Stability Study of Acid Tolerance

The same OD600 settings of bacteria solutions were prepared for green tea extractions to assess the pH stability. A-600ul of prepared LB, ST, and EC suspension

was centrifuged at 5000 rpm for 10 minutes in each of the four plastic snap-cap tubes to get bacteria pellets. The 4X 90ul of pure clementine orange juice was transferred into the bacteria tubes to make a 90ul base solution separately for treatment. 10ul of the same tea stock (5mg/ml of EGCG, P-EGCG and EGCG-S) were put into a bacterial clementine orange juice mixture as experimental groups. 10ul of freshly procured clementine orange juice were mixed into three separate 90ul of LB, ST, and EC bacterial sample as a control group. The tubes were then shaken for 60 seconds. The control and experimental groups were left in the same temperature as experiments 3 & 4 respectively. After treating, the clementine orange suspensions were diluted with DI water. Samples were plated on nutrient agar plates in a countable range. Then, the plates were left at 37 C in an incubator to grow overnight. The colonies were counted and the % of inhibition was determined. Figure 1 shows the inhibition of LB, ST, and EC activity by various catechins in vitro.

The equation of percentage of inhibition was:

$$\% \text{ of Inhibition} = \left( \frac{\text{CFU}_{\text{Control}} - \text{CFU}_{\text{Treated}}}{\text{CFU}_{\text{Control}}} \right) \times 100$$

## 6. Data Analysis

All experiments were performed in triplicate. Data are expressed as means  $\pm$  standard deviation. Statistical analysis and liner regression were performed using SSPS version 23 for Apple OS. GLM UNIANOVA and one-way ANOVA were set for data comparison. Significance was established at values of  $P < 0.05$ .

## RESULTS

For the purpose of this experiment, all these three green tea polyphenols should undergo stability testing before adding them into commercial processed foods. To evaluate

the efficacy of EGCG, P-EGCG and EGCG-S, this study tested the bacteria inhibition rate among three bacteria cultures (*L. burglarious*, *S. thermophiles*, and *E. coli*) by treating with these three tea extractions. According to the above mentioned, *E. coli* cannot survive at a higher pasteurized temperature (68°C- 73°C) (U.S. Food and Drug Administration, 2014). In addition, there was no *E. coli* bacteria growth in a 68 C environment in the study (Fig. 2). As it has been well proven that *E. coli* does not survive in a pasteurized environment at temperatures above 68°C, this bacterium were not considered in the present study in a pasteurized condition. The results from the CFU study are shown in Figure 3, and 4 for EGCG, P-EGCG, and EGCG-S.

#### 1. The Effect of Tea Polyphenols in Nutrient Broth

The stability of EGCG, P-EGCG, and EGCG-S were determined by LB, ST, and EC. The stability profiles from the CFU study are shown in Figs. 3A, 3B and 3C, respectively. At room temperature (27°C, Fig. 3A), all three green tea extractions only marginally restricted LB growth (EGCG 40%, P-EGCG 45%, EGCG-S 40%). The high percentage of inhibition of ST activity caused by EGCG, P-EGCG, and EGCG-S was 92.7%, 96.4%, and 96.7%, respectively. EGCG (94.1%), P-EGCG (98.7%) had better percentage of inhibition of EC than EGCG-S. P-EGCG and EGCG-S inhibited more than 98% of three types of bacteria growth at a 37°C (Fig. 3B) condition, while EGCG was much less effective at prohibiting LB (25.1%), ST (18.5%), and EC (43.6%) bacteria growth. In the 55°C CFU study, LB was almost completely inhibited by treating with all tea solutions. The percentages of inhibition of P-EGCG, EGCG-S were over 99%, and 93% respectively for all bacteria in the study (Fig. 3C). Bacterial inhibition by P-EGCG and EGCG-S solutions were both above 99% at experiment 2a (24 h 68 C water bath incubate, Fig. 3D) condition.



However, EGCG incubated for the overnight 2a high temperature condition negatively affected its antibacterial effectiveness in LB and ST. ST showed negatively constrained growth in an EGCG incubation. In the experiment 2b (bacterial tea solutions pre-water bath at 68°C for 30 min before 24h 37°C incubation, Fig. 3E) condition, the percent of inhibition of LB, ST caused by P-EGCG and EGCG-S were both over 95%. P-EGCG, and EGCG-S showed high percentages of inhibition of around 95% in LB, and ST of experiment 2c (bacterial tea solutions re-water bath at 68°C for 30 min after 24h 37°C incubation, Fig. 3F) environment. A significant decrease of ST bacteria inhibition activity was observed in EGCG, (81.8%) for this condition.

## 2. Stability Comparison in Different Temperature (pH=7)

EGCG, P-EGCG, and EGCG-S were measured in two different experimental conditions when pH = 7.0: in experiment 1 the incubation period for all green tea extractions did inhibit bacteria growth (Fig. 3). Bacteria type had no influence on the green tea polyphenols' effectiveness in the study. The factors which influence the percentage of bacteria growth during the experiment 1 (at 25°C, 37°C, and 55°C) were pooled together as shown in Table 1. As can be seen in Table 1, the differences among the three green tea extractions for experiment 1 were significant ( $P < 0.05$ ). In addition, when the temperature increased, P-EGCG, and EGCG-S showed positive results constraining growth of LB, ST, and EC. EGCG was unstable in different temperature conditions inhibiting bacteria (Table 1). For conditions in experiment 2, there was no significant difference of bacteria inhibition of P-EGCG, EGCG-S (Table 2). There was a significant difference ( $P < 0.05$ ) of the bacteria inhibition of P-EGCG for all of experiment 2 temperature incubation conditions. Only the green tea extracts were a major factor influencing the inhibition rate of bacteria.

The various pasteurized processes (at 68 C) or bacterial types did not produce significantly differences ( $P > 0.05$ ). As referenced in Table 1 and Table 2, green tea extractions, especially P-EGCG, had a significant difference when comparing stability in different temperature conditions.

Table 1: Three bacteria (LB, ST, EC) mixing nutrient broth by three tea extractions treating at room temperature (27°C), 37°C, and 55°C conditions. Values are the mean of three experiments.

temperature	bacteria type	green tea extractions	% of inhibition
25	LB	EGCG	40
25	LB	P-EGCG	45
25	LB	EGCG-S	40
37	LB	EGCG	25.1155624
37	LB	P-EGCG	99.9745763
37	LB	EGCG-S	99.6779661
55	LB	EGCG	100
55	LB	P-EGCG	99.980392
55	LB	EGCG-S	100
25	ST	EGCG	92.71162123
25	ST	P-EGCG	96.41319943
25	ST	EGCG-S	96.67144907
37	ST	EGCG	18.442623
37	ST	P-EGCG	99.3545082
37	ST	EGCG-S	98.5297131
55	ST	EGCG	31
55	ST	P-EGCG	99.75
55	ST	EGCG-S	93.125
25	EC	EGCG	94.04444444
25	EC	P-EGCG	98.74444444
25	EC	EGCG-S	53.33333333
37	EC	EGCG	43.5684647
37	EC	P-EGCG	99.9118257
37	EC	EGCG-S	99.9792531
55	EC	EGCG	71.31147541
55	EC	P-EGCG	99.50810672
55	EC	EGCG-S	99.67213115
pH=7.0		% of inhibition (p value)	
temperature (25°C, 37°C, 55°C)		0.515	
bacteria type (LB, ST, EC)		0.673	
green tea extractions (EGCG, P-EGCG, EGCG-S)		0.014	

Table 2: Bacteria mixed nutrient broth, using three tea extractions to treat at the pasteurized temperature (68°C) for 24h, 30 mins, 30 mins +24h (room temperature). % of inhibition values are the mean of three experiments.

<b>pasteurized manipulate</b>	<b>bacteria type</b>	<b>green tea extractions</b>	<b>% of inhibition</b>	<b>analysis of Variation</b>	<b>P value</b>
<b>pasteurized 24 h</b>	LB	EGCG	38.303342	EGCG	0.158
		P-EGCG	100	P-EGCG	0.03
		EGCG-S	100	EGCG-S	0.081
	ST	EGCG	-74.86	LB	0.366
		P-EGCG	100	ST	0.307
		EGCG-S	99.0357143	pasteurized 24 hrs.	0.13
<b>pasteurized 30 min before room temperature treating</b>	LB	EGCG	95.5351213	pasteurized 30 min before room temperature treating	0.473
		P-EGCG	97.0268199		
		EGCG-S	97.0472547		
	ST	EGCG	89.2670157	pasteurized 30 min after room temperature treating	0.489
		P-EGCG	95.0366492		
		EGCG-S	94.408377		
<b>pasteurized 30 min after room temperature treating</b>	LB	EGCG	95.6124031	/	
		P-EGCG	94.2635659		
		EGCG-S	96.4186047		
	ST	EGCG	81.829633		
		P-EGCG	95.6845426		
		EGCG-S	95.0536278		

### 3. The Effect of Tea Polyphenols in Clementine Orange Juice

The stability of EGCG, P-EGCG, and EGCG-S in clementine orange juice, pH 3.03, for conditions in experiments 3&4, were determined. The stability profiles of EGCG, P-EGCG, and EGCG-S are shown in Figs. 4. All bacteria were relatively resistant to homemade clementine orange juice. In the conditions for experiment 3 (Fig. 4A, 4B, 4C), P-EGCG and EGCG-S inhibited more than 90% of LB growth, while the percentage of the inhibition of EGCG (15.2%) was significantly lower than P-EGCG and EGCG-S at room temperature (25°C). CFU findings of ST showed that P-EGCG, and EGCG-S were more

effective than EGCG in inhibiting bacteria growth. However, at 24 h 37°C, all three green tea extractions inhibited ST less than 40%.

EC colony counts demonstrated that P-EGCG and EGCG-S were more effective in inhibiting bacteria growth at 24 h 37°C. The percent of inhibition of EC were over 95% for both polyphenols. As the temperature was increased, for the three conditions 25°C, 37°C, & 55°C, the inhibition rate of EGCG decreased. In addition, at 24 h 37°C incubated condition, EGCG was ineffective at reducing EC. Treatment of P-EGCG, and EGCG-S concentration completely eliminated LB, and ST (100% reduction,  $p < 0.05$  to  $p < 0.001$ ) from 24 h water bath condition (Fig. 4D). P-EGCG, and EGCG-S caused an over 95% LB inhibition in experiment 4b (bacterial tea solutions pre-water bath at 68°C for 30 min before 24h 37°C incubation) condition. P-EGCG and EGCG-S caused greater LB, and ST inhibition than EGCG in all experiment 4 conditions.

#### 4. Stability Comparison in Different Temperatures (pH=3.03)

There was a large different of LB, ST and EC inhibition expressed in experiment 3 using EGCG. The percentage of bacteria inhibition of EGCG was lower than P-EGCG, and EGCG-S. The differences among the three green tea extractions for bacteria inhibition were significant in the experiment 3 environment ( $P < 0.05$ ). Tea polyphenols were the most important factors affecting bacteria growth (LB, ST, EC). There was no significant difference of bacteria inhibition when the incubated temperature was increased in experiment 3 conditions. Possible effects of the amount and pattern of bacteria growth were assessed by GLM UNIANOVA analyses. Bacterial inhibition test conditions of temperature, bacterial types and green tea extraction variant were conducted in isolation (Table 3).

**Table 3:** Three bacteria (LB, ST, EC) mixing orange juice by three tea extractions treating at room temperature (27°C), 37°C, and 55°C conditions. Values are the mean of three experiments.

Temperature (°C)	Bacteria Type	Green Tea Extractions	% of inhibition
25	LB	EGCG	15.2173913
25	LB	P-EGCG	96.4285714
25	LB	EGCG-S	95.9627329
37	LB	EGCG	95
37	LB	P-EGCG	91.25
37	LB	EGCG-S	91.25
55	LB	EGCG	95.3488372
55	LB	P-EGCG	100
55	LB	EGCG-S	100
25	ST	EGCG	74.0740741
25	ST	P-EGCG	81.1111111
25	ST	EGCG-S	79.5555556
37	ST	EGCG	22.5
37	ST	P-EGCG	22.1428571
37	ST	EGCG-S	38.9285714
55	ST	EGCG	91.0791367
55	ST	P-EGCG	100
55	ST	EGCG-S	100
25	EC	EGCG	60.4878049
25	EC	P-EGCG	99.1463415
25	EC	EGCG-S	99.329268
37	EC	EGCG	-51.052632
37	EC	P-EGCG	95.7894737
37	EC	EGCG-S	97.8947368
55	EC	EGCG	1.96078431
55	EC	P-EGCG	100
55	EC	EGCG-S	100
Research factors		Effect of amount consumed	
temperature (25°C, 37°C, 55°C)		$F = 2.148, p = 0.143, \text{Eta-squared} = 2364.404$	
bacteria type (LB, ST, EC)		$F = 1.019, p = 0.379, \text{Eta-squared} = 1122.073$	
tea extractions (EGCG, P-EGCG, EGCG-S)		$F = 5.118, p = 0.016, \text{Eta-squared} = 5635.034$	

Experiment 4 was carried out to determine the stability effects of three green tea extractions. The differences among the three green tea extractions for experiment 4 environments were significant ( $P < 0.05$ ). There was no significant difference of bacteria inhibition of bacteria type (Table 4). Pasteurization did not result in significant changes of stability of green tea polyphenols.

**Table 4:** Three major factors which can influence the percentage inhibition of bacteria in clementine orange juice pasteurized processing condition. The mean scores indicate moderately positive perceptions of the effects of green tea polyphenols on bacteria inhibition.

SOURCE	SD	P VALUE
pasteurized manipulating (a, b, c)	1.244	0.323
tea polyphenols (EGCG, P-EGCG, EGCG-S)	4.033	0.046
bacteria (LB, ST)	0.008	0.929

*\*a expressed as pasteurized 24 hrs.*

*\*b expressed as pasteurized 30 min before room temperature treating*

*\*c expressed as pasteurized 30 min after room temperature treating*

*\*SD Standard deviation*

Treatment of P-EGCG inhibited LB, ST, and EC cells ranged from 66 to 100%, 22 to 100% and 95 to 100% respectively, at all the temperature points. The percentage of inhibition of EGCG-S ranged from 38 to 100% (LB), 29 to 100% (ST) and 97 to 100% (EC) respectively, at experiment 3 & 4 temperature studies. These observations also indicated that the inhibitory effect of P-EGCG, and EGCG-S were significantly greater ( $p < 0.01$ ) than that of EGCG treatment.

## DISCUSSION

Several studies reported that many factors (eg: pH, temperature, antioxidant level) may affect the stability of EGCG (Z. Chen, Zhu, Tsang, & Huang, 2001; Hou et al., 2005; Labbé, Têtu, Trudel, & Bazinet, 2008; Su, Lai, Huang, & Chen, 2003; Wolfram, Wang, & Thielecke, 2006). Comparing the data from Table 1 & 3, the results support that EGCG is more stable in an acidic pH environment than alkaline environment (Kumaran, Arulmathi, Srividhya, & Kalaiselvi, 2008; Pollard et al., 2009; Zhong & Shahidi, 2012). The present study compares the stability of EGCG, and two more EGCG modifications (P-EGCG, EGCG-S) at different temperature and pH conditions. The GLM UNIANOVA analyses (Table. 5) reveals a positive significant difference of EGCG when comparing the effect with P-EGCG and EGCG-S in experiment 1 & 3 ( $p = 0$ ,  $p = 0.001$ ) conditions, the results support that EGCG was unstable under higher- temperature environments (Hirun & Roach, 2005). In experiment 2 & 4 conditions, there are significant differences between different pH, and different green tea extractions. Polyphenol types and pH levels had very small interactions in these pasteurized study conditions ( $p > 0.05$ ) (Table. 6). In all stability research conditions, green tea extractions are the major factors that inhibit bacteria growth. In addition, P-EGCG, and EGCG-S are more effective in inhibiting bacteria growth in acidic pH conditions than EGCG. The data in the clementine orange juice environment study agrees with the result that an acidic pH around 4.0 could help the catechins to limit degradations (Bazinet, Araya-Farias, Doyen, Trudel, & Têtu, 2010). For all experiments, bacterial inhibition test conditions of temperature, bacterial types and GTE variants were conducted in isolation.



Table 5: Compare the bacteria inhibition rate of three green tea extracts in different pH value conditions. The mean scores indicate the effects of green tea polyphenols on bacteria inhibition at different pH levels (pH=7, pH=3.03), ( $P < 0.05$ ,  $P < 0.01$ ).

	Effective Comparison		Mean Difference	<i>p</i> Value
Temperature (25°C, 37°C, 55°C)	EGCG	P-EGCG	-39.094	0
	EGCG	EGCG-S	-36.839	0.001
	P-EGCG	EGCG-S	2.255	0.825
Pasteurized Manipulating (a, b, c)	EGCG	P-EGCG	-96.397	0.005
	EGCG	EGCG-S	-93.573	0.006
	P-EGCG	EGCG-S	2.824	0.929
	pH=3.03	pH= 7.0	-53.535	0.046

*\*a expressed as pasteurized 24 hrs.*

*\*b expressed as pasteurized 30 min before room temperature treating*

*\*c expressed as pasteurized 30 min after room temperature treating*

Table 6: Three green tea extracts, pH levels, and different temperature processing as the main factors to impact the bacteria inhibition in Experiment 1 & 3, and Experiment 2 & 4.

Research Condition	Major Factor	Mean (SD)	Effect of Amount Consumed
Temperature (25°C, 37°C, 55°C) Experiment 1 & 3	EGCG	51.156 (41.837)	$F=9.411, p=0$
	P-EGCG	90.250 (21.450)	
	EGCG-S	76.467 (34.325)	
Pasteurized Manipulating (a, b, c) Experiment 2 & 4	EGCG	-7.332	$F=6.072, p=0.006$
	P-EGCG	89.065 (17.221)	
	EGCG-S	86.241 (24.629)	
	pH=3.03	29.224 (119.524)	$F=4.337, p=0.046$
	pH= 7.0	82.759 (41.783)	
	pH * tea polyphenol	55.992 (92.325)	$F=1.842, p=0.176$

\*SD Standard deviation

\*a expressed as pasteurized 24 hrs.

\*b expressed as pasteurized 30 min before room temperature treating

\*c expressed as pasteurized 30 min after room temperature treating

The results from the present study of EGCG are in agreement with the findings obtained in previous research (Cui et al., 2012; Hirun & Roach, 2005; Sakanaka et al., 1989). The current temperature study supports that the shelf life of catechins could be extended in a lower temperature environment. Also EGCG positively affected stability at a lower storage temperature (4 °C) (Bazinet et al., 2010; Jodoin, Demeule, & Beliveau, 2002). The present study demonstrates that P-EGCG, EGCG-S at all temperatures and acid conditions are stable for at least 24 h. Comparing the results from EGCG, P-EGCG, and EGCG-S, the percentage of inhibition of EGCG is lower than P-EGCG, and EGCG-S for

LB, ST at pH=7, or pH=3.03 bacterial tea solutions for different water bath pasteurized processes. Green tea polyphenols, temperature conditions, pH conditions, bacteria types are the four main factors in the current research. The GLM UNIANOVA analyses show that bacteria types and temperature conditions were the smallest effective factors for bacteria inhibition. The results indicated that P-EGCG and EGCG-S are more stable than EGCG at acidic and neutral values in a higher temperature environment. While all three green tea extractions can be considered as food additives in acidic and neutral food products (R. Wang & Zhou, 2004), P-EGCG has the best potential for reduction of bacterial growth. P-EGCG is considerably more resistant at higher temperatures and lower pH values. Therefore, P-EGCG could be considered the most stable green tea extraction to inhibit foodborne bacteria growth.

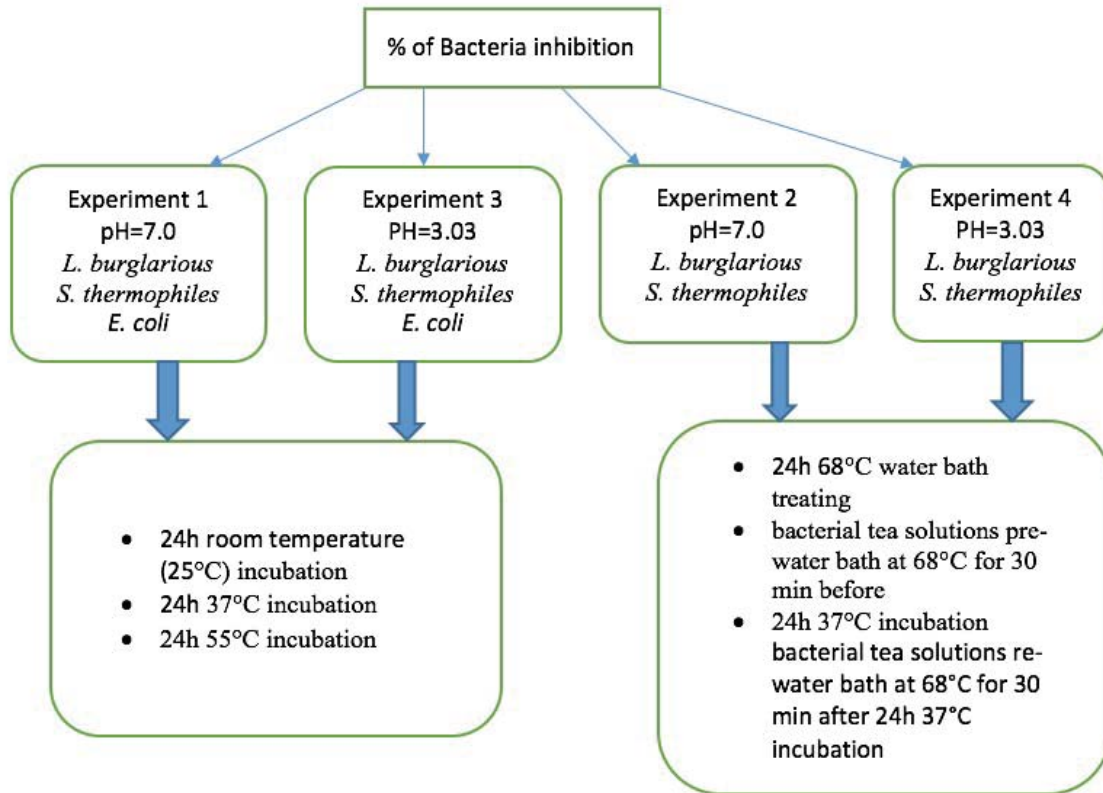
## **ACKNOWLEDGEMENTS**

This research was supported by the Chemistry and Biochemistry and Nutrition and Food Studies laboratories at Montclair State University, NJ.

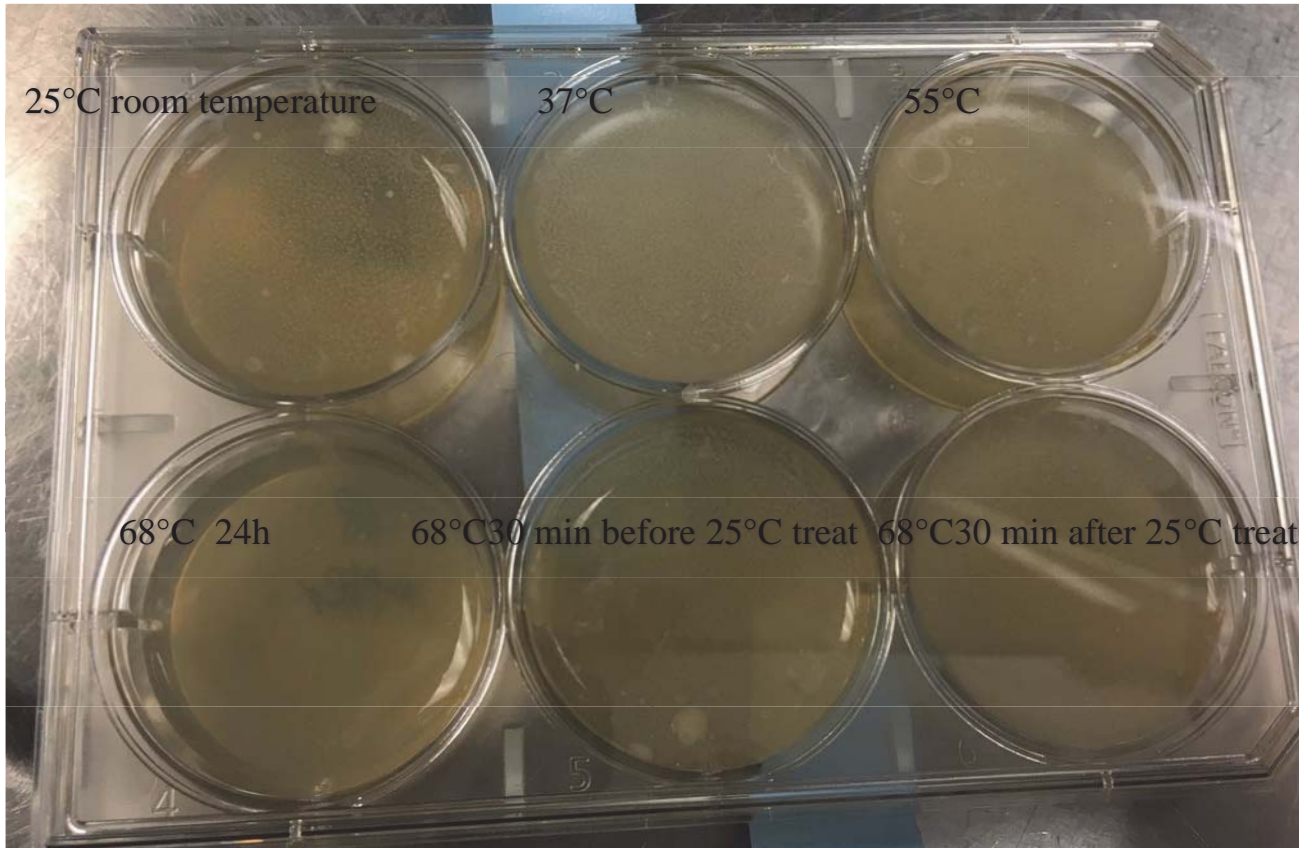
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## FIGURES:

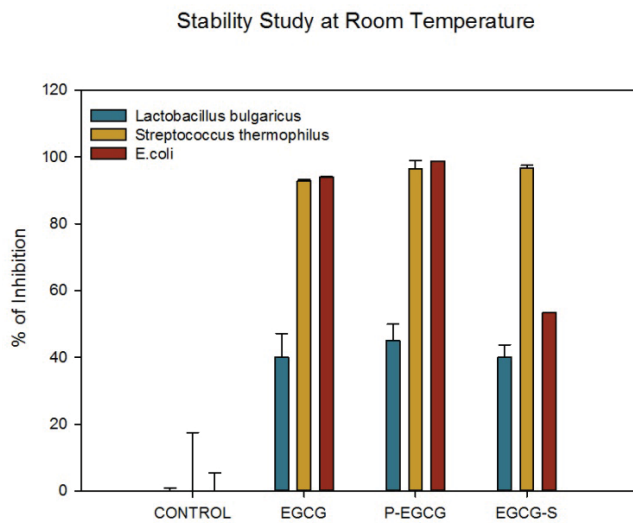
**Figure 1.** four experiments of EGCG, P-EGCG, and EGCG-S stability study detail.



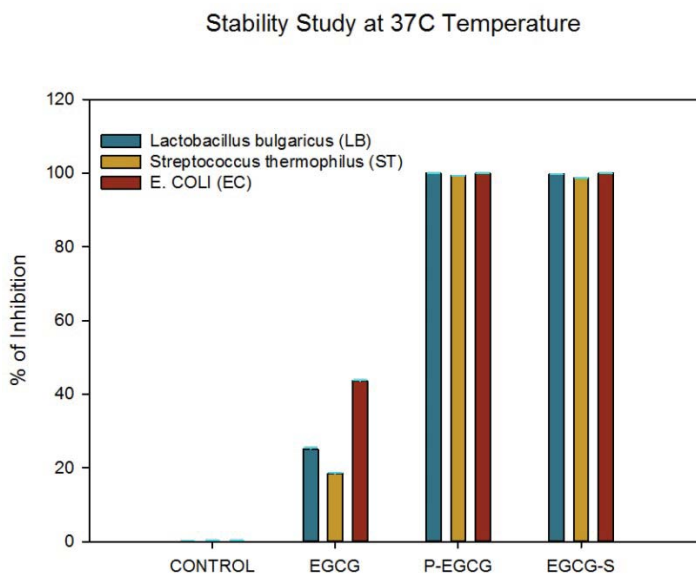
**Figure 2.** *E. coli* grow overnight in nutrient agar 37°C incubation at different temperature conditions (25°C, 37°C, 55°C, 68°C).



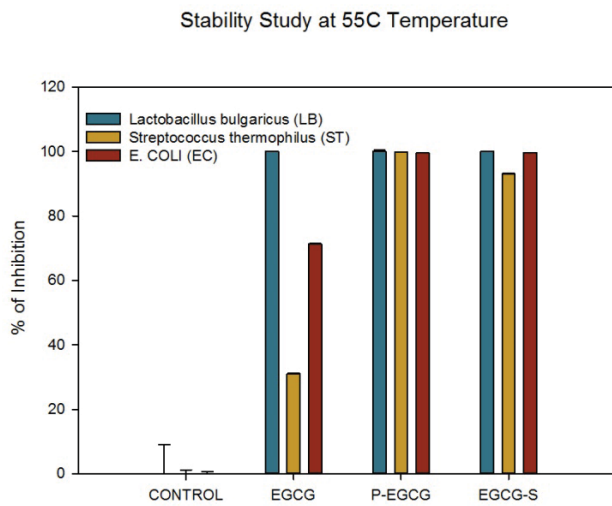
**Figure 3A.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 25°C room temperature treating in nutrient agar (n=3).



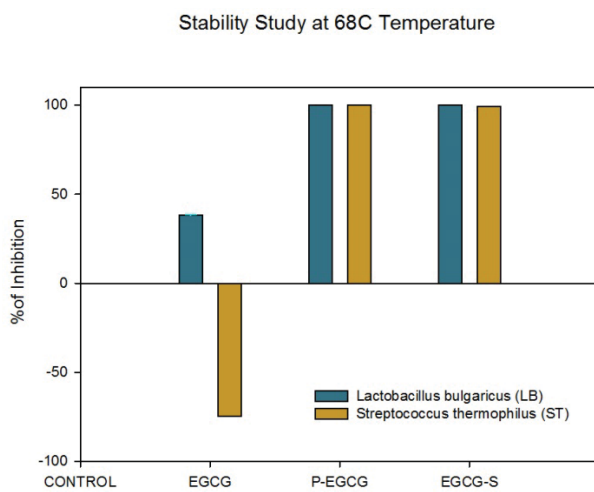
**Figure 3B.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 37°C temperature condition in nutrient agar (n=3).



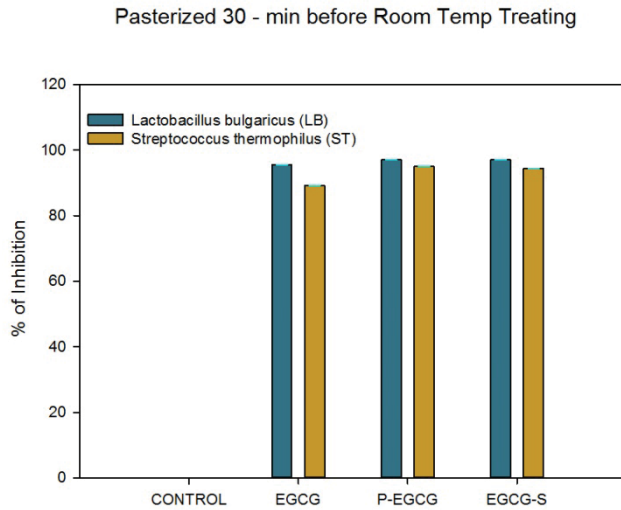
**Figure 3C.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 55°C temperature condition in nutrient agar (n=3).



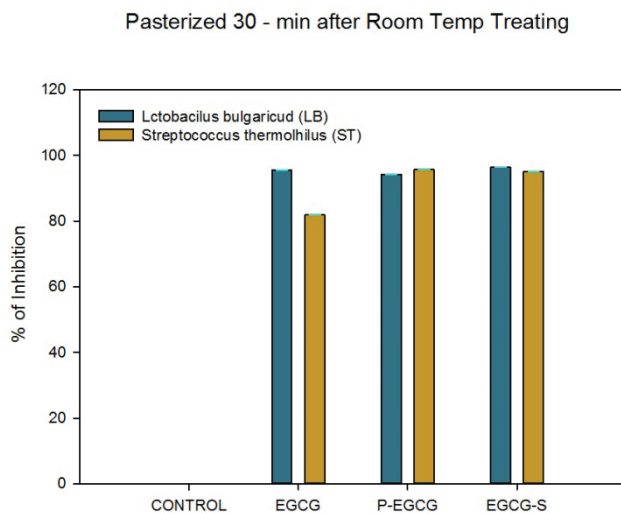
**Figure 3D.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 24 hours' 68°C temperature water bath treating in nutrient agar (n=3).



**Figure 3E.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 68°C in 30 minutes before treat the bacteria in room temperature one day in nutrient agar (n=3).

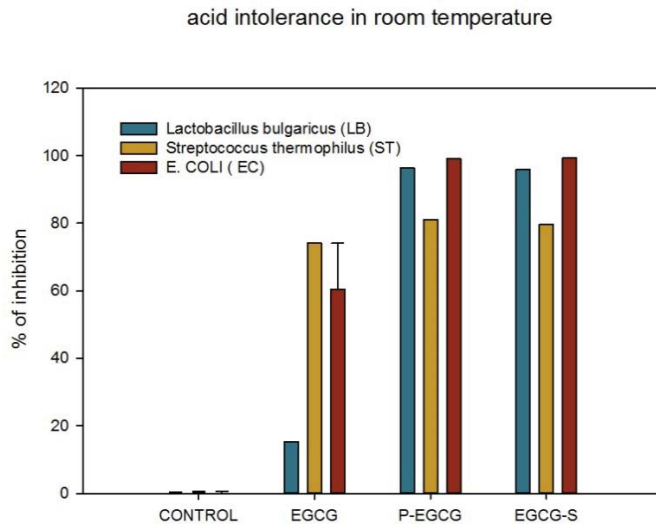


**Figure 3F.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 68°C in 30 minutes after treat the bacteria in room temperature one day in nutrient agar (n=3).

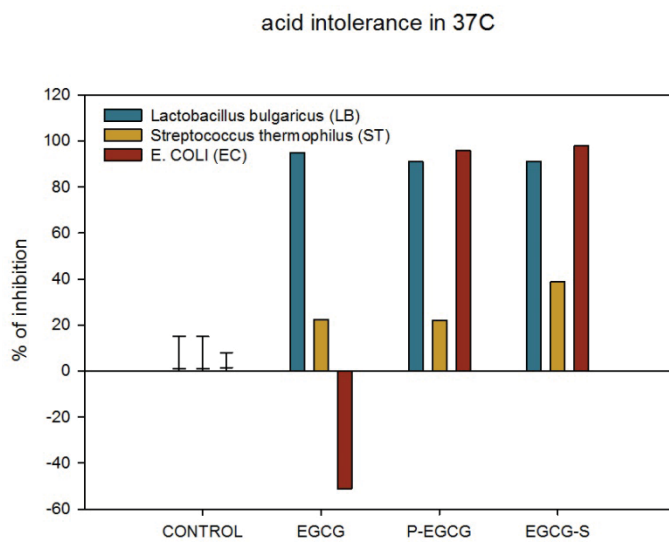




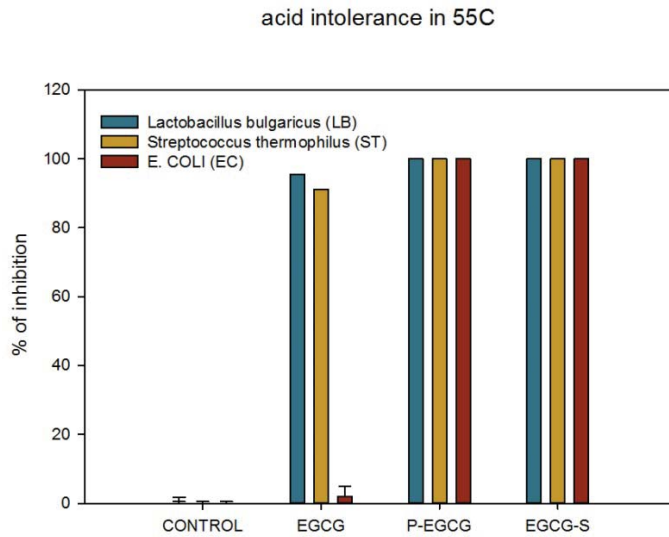
**Figure 4A.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 25°C room temperature treating in clementine orange juice (n=3).



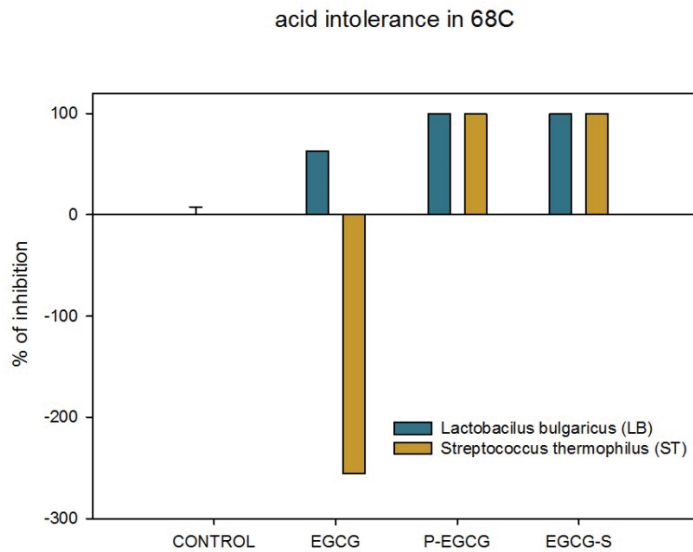
**Figure 4B.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 37°C temperature condition in clementine orange juice (n=3).



**Figure 4C.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 55°C temperature condition in clementine orange juice (n=3).



**Figure 4D.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 24 hours' 68°C temperature water bath treating in clementine orange juice (n=3).



Manuscript #1 is the preliminary study of manuscript #2. In manuscript #1, the research study indicated the stability of epigallocatechin-3-gallate (EGCG), epigallocatechin-3-gallate-sterate (EGCG-S), and palmitoyl-epigallocatechin-3-gallate (P-EGCG) in various temperature and pH conditions. The results showed that P-EGCG and EGCG-S are more stable than EGCG in a higher temperature, and lower pH condition. As food preservatives, whether these three green tea extractions would influence the nutrient value in processed food is a valuable question to be researching. Thus, in manuscript #2, the study used vitamin C as a research nutrient factor to evaluate whether EGCG, P-EGCG, and EGCG-S would destroy the vitamin C content and antioxidant ability in clementine orange juice.

## **MANUSCRIPT #2**

**Running title:** Green tea polyphenols for retaining antioxidant and vitamin C properties

### **Three green tea polyphenol's potential for retaining antioxidant and vitamin C properties in clementine orange juice**

Danxia Shi BS<sup>1\*</sup>, Charles Feldman PhD<sup>1</sup>, Chia-liang Huang BS<sup>1</sup>,  
Shahla Wunderlich PhD<sup>1</sup>, Lee Lee PhD<sup>2</sup>

<sup>1</sup>Montclair State University, Department of Nutrition and Food science; and <sup>2</sup>Department of Biology, NJ 07043, USA

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\*Author for correspondence. Tel: (862) 213-1573; E-mail: shid3@montclair.edu

## RESEARCH HIGHLIGHTS

► Epigallocatechin-3-gallate-stearate (EGCG-S), and palmitoyl-epigallocatechin gallate (P-EGCG) were more stable than epigallocatechin-3-gallate (EGCG) at acid (pH3.03) and pasteurized temperature (68°C) conditions. ► P-EGCG and EGCG-S inhibited more testing bacteria in the control conditions ► The extracts did not affect the vitamin C content in clementine orange juice at specific control temperatures ► P-EGCG had best activity than others for reducing the oxidant rate of vitamin C in the clementine orange juice.

## ABSTRACT

Green tea is an excellent food source for dietary consumption, and it has the potential for future use as a food preservative. It is grown in over 30 countries. Epigallocatechin-3-gallate (EGCG) is one of the major polyphenols in green tea. It has antioxidant properties as a natural food additive, and can potentially store food longer and fresher. The present study investigates the stable and antioxidant abilities of three green tea polyphenols: epigallocatechin-3-gallate (EGCG), epigallocatechin-3-gallate-stearate (EGCG-S), and palmitoyl-epigallocatechin gallate (P-EGCG). This investigation utilized clementine orange juice as a baseline for vitamin C concentration levels (pH3.03). The results showed positive correlations for preservation and nutrient retention under pasteurized temperature (68°C) and high-acid conditions (pH3.03). Using a 30-minute water bath pretreatment at 68°C, and a 24-hour incubation at 25°C, P-EGCG and EGCG-S inhibited over 95% of *Lactobacillus burglarius* (*L. burglarius*) and 79% of *Streptococcus thermophiles* (*S. thermophiles*). Furthermore, these three polyphenols did not affect the vitamin C concentration in clementine orange juice at room temperature (25°C), or pasteurized temperature (78°C, 15 second) conditions. The antioxidant efficiencies of the homemade clementine orange juice were 95.605% (control condition), 93.8% (EGCG), 99.075% (P-EGCG) and 97.4125% (EGCG-S) respectively.

**Key words:** Green tea polyphenols; Pasteurized temperature; Low pH; Stable assessment; Clementine orange juice; Vitamin C content

## INTRODUCTION

Green tea of different varieties has been grown in over 30 countries. For centuries consumption of green tea has primarily been in China, Japan, a few countries in North Africa and the Middle East (Sakakibara, Honda, Nakagawa, Ashida, & Kanazawa, 2003). Presently, people use green tea widely for weight loss, inflammatory bowel disease, exercise enhancement; iron overload, hair loss, diabetes, and skin disorders (Balsaraf & Chole, 2015; Jankun, Selman, Swiercz, & Skrzypczak-Jankun, 1997; Sinija & Mishra, 2008). Green tea has been demonstrated to have anti-carcinogen, anti-inflammatory, anti-bacterial, and anti-viral properties (QL Dai, Xu, Lin, Wang, & Yang, 2008). Green tea has a complex chemical composition, which has proteins, vitamins (B, C, E), and elemental minerals (Ca, Mg, Cr, Mn, Fe) (Cabrera, Artacho, & Giménez, 2006).

Most of the health benefits of teas come from the antioxidant content of *camellia sinensis*. Research has demonstrated that the level of antioxidants in one to two cups of tea would be approximately equal to five times the amount found in natural fruits and vegetables (du Toit, Volsteedt, & Apostolides, 2001). Green tea is a unique antioxidant nutrient which not only enriches polyphenols, but it also contains ascorbic acid (vitamin C). These compounds in green tea could increase the potential antioxidant ability of green tea polyphenols (Cabrera et al., 2006). Catechins also have been reported to show high antioxidant levels in vitro and in vivo (Balsaraf & Chole, 2015). The four main tea flavonoids in catechins are epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), and epigallocatechin-3-gallate (EGCG)(Cabrera et al., 2006; Sinija & Mishra, 2008). EGCG (chemical structure Fig. 1) is the most abundant and active catechin among all of these epicatechins (Lakenbrink et al., 2000; H. Wang & Helliwell, 2000).

Moreover, EGCG has shown to be effective in inhibiting gram-positive bacteria growth (*S. mutants* and *S. aureus*) (Cui et al., 2012; Sakanaka et al., 1989).

The antioxidant and bioactive ability in green tea are dependent on the absorption, distribution, metabolism, and excretion (ADME) properties of catechin (Balsaraf & Chole, 2015; Widyaningrum et al., 2006). EGCG has high antioxidant properties when used as a natural food additive and in clinical treatments (Kumaran et al., 2008; Pollard et al., 2009; Zhong & Shahidi, 2012). The synthesis of benzothiophene derivatives of EGCG has higher AOA levels than EGCG by itself (Pollard et al., 2009). Epigallocatechin-3-gallate-stearate (EGCG-S) (Figure 2), and palmitoyl-epigallocatechin gallate (P-EGCG) are modified versions of the primary molecule EGCG. In a recent study, the modified EGCG, P-EGCG, showed effective inhibition of herpes simplex virus 1 (HSV-1) infections (De Oliveira et al., 2013). Furthermore, EGCG and P-EGCG have been approved as safe additives for food in China. Thus, EGCG, P-EGCG, and EGCG-S are potential green tea extractions to be considered for food protection in future food science studies and applications.

Vitamin C is an essential vitamin and is important as a component of the overall antioxidant protective mechanisms in cells and tissues as many diseases are related to cell oxidation. The high availability of antioxidants in vitamin C could help people decrease the likelihood of disease and provide a defense against pathogen invasions (Bendich et al., 1986; Carr & Frei, 1999; Jacob & Burri, 1996; C. Kaur & Kapoor, 2001; Podsędek, 2007; Schwartz & Weiss, 1994). Epidemiological studies have provided overwhelming evidence demonstrating that fruit and vegetable daily dietary intake is related to reduction of the risk of degenerative diseases (Cao, Booth, Sadowski, & Prior, 1998; Nicoli, Anese, & Parpinel, 1999), because most vegetables and fruits contain vitamin C, particularly oranges.



Polyphenols and vitamin C in fruits are the major contributors for the food's antioxidant capacity (Du et al., 2009). The AOA of citrus fruits and juices are important to human nutrition (Ghasemi, Ghasemi, & Ebrahimzadeh, 2009). Citrus juices including oranges (*citrus sinensis*) and tangerine (*citrus reticulata*) varieties are the most popular juices consumed in the US (Vinson et al., 2002). The AOA of citrus juices depend on their genetic variety (Kelebek, Selli, Canbas, & Cabaroglu, 2009). General orange juice contributes a high amount of total phenolic and antioxidants (146.6 mg of vitamin C) in the American common diet (Chun et al., 2005). Clementine oranges are a smaller version orange among *citrus sinensis* varieties. They are sweeter and juicier than regular orange (Wu et al., 2014). In addition, clementine oranges have been found to contain abundant vitamins (vitamin C, vitamins B1, B2, and B3) and microelements (potassium, magnesium, and zeaxanthin) (Ladaniya & Ladaniya, 2010). In the United States, processed orange production comes mostly from California, Texas and Alabama (Burton, 2017). Multiple studies demonstrated that clementine oranges have high antioxidant ability that is mainly associated with vitamin C (Álvarez et al., 2012; Gardner et al., 2000; Pellegrini et al., 2003; Sdiri, Navarro, Monterde, Benabda, & Salvador, 2012). Therefore, clementine oranges are a good sample for a prospective study on the efficacy of green tea polyphenols for retaining of antioxidant and vitamin C properties.

High hydrostatic pressure (500 Mpa, 35 °C, 5 min) and pasteurization of orange juice can help the juice extend its shelf life at temperatures below 5°C (Polydera et al., 2003), the longer citrus juice is stored, the potency of vitamin C decreases (Cortés, Esteve, & Frígola, 2008; H. Lee & Coates, 1999; Yeom, Streaker, Zhang, & Min, 2000). A recent study suggest the addition of pasteurized tea extracts could enhance the antioxidant

properties of processed orange juice (Qi Dai, Borenstein, Wu, Jackson, & Larson, 2006), however this has get to be tested at high ( pasteurization) temperature.

In consideration of the findings in the literature on the efficacy of EGCG as it is already being used as a preservative in the field, we hypothesize that P-EGCG, and EGCG-S could effectively be utilized as an additive in clementine orange juice to retain vitamin C and antioxidant properties at different temperatures.

## **MATERIALS AND METHODS**

The present study used *Lactobacillus burglariious* and *Streptococcus thermophiles* to address whether EGCG, P-EGCG, and EGCG-S are stable at pasteurized temperatures and low PH conditions. The investigation was focused on the antioxidant capacity of EGCG, P-EGCG, and EGCG-S. For the purposes of this study, different temperature conditions were considered in pure clementine orange juice made from fresh clementine orange in laboratory conditions. Vitamin C was used as a marker to analyze the optical density using UV 246nm. Additionally, EGCG, P-EGCG, and EGCG-S were tested for antioxidant concentrations of vitamin C in the clementine orange juice.

### **Strains and culture preparation.**

*L. burglariious* and *S. thermophiles* cultures, isolated and purified from homemade yogurt were maintained in nutrient agar (Difco™) plates. The pure bacterial stock cultures were grown overnight and the purity of the cultures were determined before each experiment. If bacterial contamination was observed, the discontinuous streaking method was used to isolate a single purified colony. The overnight cultures obtained from the original nutrient agar plates were incubated at 37 °C for 24 hours. The cultures were then transferred to the nutrient broth (Difco™) to get the optical density of 0.1 at OD 600 nm.

### **Utilization of three green tea polyphenols.**

Three different tea extractions: epigallocatechin-3-gallate (EGCG), palmitoyl-epigallocatechin gallate (P-EGCG), and epigallocatechin-3-gallate-stearate (EGCG-S) were tested in the experiment. Then, all tea compounds were prepared at the final concentration of 5mg/ml as polyphenol stocks.

### **Clementine Orange Juice Preparation.**

Clementines (*citrus reticulata*) were procured for experimentation on January 18, February 20, and March 3, 2017. These particular "Darling Clementines," grown in California and exclusively trademarked and marketed by LGS Specialty Sales, New Rochelle, NY, were purchased at Shoprite in Bloomfield, NJ. The clementine orange juice was prepared, after suitable washing and a 10-minute ultraviolet radiation sterilization in the Montclair State University Biology Laboratory. The fresh clementine oranges were squeezed to produce juice in a flask. The pH of the sample juice was then tested.

### **Agar and broth Preparation.**

Nutrient agar (Difco™) was prepared by 30 grams of agar powder and 1 liter of deionized (DI) water. The media was thoroughly mixed before autoclaving at 121°C for 15 to 20 minutes. Then, the mixture was poured into sterile plates and were left to solidify at room temperature. After solidifying, the nutrient agar plates were exposed to ultraviolet light for 20 minutes. The plates were then safely stored in a cold room at 4°C.

The broth solution was prepared by mixing 18 grams of nutrient broth (Difco™) with 1 liter of DI water for preparing a broth solution. The solution was autoclaved at 121°C for 15 to 20 minutes. Then the media was cooled down to room temperature before being stored in a cold room at 4°C.

### **Stability studies: EGCG, P-EGCG and EGCG-S in different environments.**

CFU assay was used for determining the viability of bacterial colonies in the stability study.

OD600 of 0.1 of bacteria broth was prepared for green tea extractions for acid testing. A 600ul of prepared bacteria broth was centrifuged in each of the four tubes at 5000 rpm for 10 minutes to get bacteria pellets. The 4X 90ul of pure clementine orange juice was transferred into the bacteria tubes to make a 90ul base solution separately for treatment. 10ul of tea stock (5mg/ml of EGCG, P-EGCG and EGCG-S) were put in clementine orange + bacteria solution for the experiments. 10ul of original clementine orange juice was placed into a 90ul bacterial mixture of clementine orange juice as a control. The tubes were then shaken for 60 seconds. The control and experiment groups were left at room temperature and then placed in a 68°C water bath for durations of 30 mins, and 24 hours respectively. After treating, a serial dilution was performed with DI water for both the control and experiment groups. Samples were plated on nutrient agar plates in a countable range. Then, the plates were left at 37°C in an incubator to grow overnight. The colonies were counted and % of inhibition were determined.

The equation of percentage of inhibition was:

$$\% \text{ of Inhibition} = \left( \frac{\text{CFU}_{\text{Control}} - \text{CFU}_{\text{Treated}}}{\text{CFU}_{\text{Control}}} \right) \times 100$$

### **Ascorbic acid determination.**

The spectrophotometric method utilized followed procedures previously employed in multiple investigations (Garnero & Longhi, 2007; Parthasarathy & Nandakishore, 2014; Takashima, Shichiri, Hagihara, Yoshida, & Niki, 2012). The determination of ascorbic acid

(AA) was performed by using a 10S UV-Vis Spectrophotometer (GENESYS™) at a wavelength of 246 nm.

#### Standard ascorbic acid solution

0.0252g standard crystalline ascorbic acid was dissolved into a mixture of acid reagents. Then, DI water was added to prepare 250ppm standard stock solution.

#### Mixture acid reagents

3ml of 3% H<sub>3</sub>PO<sub>4</sub> reagent was mixed with 7ml of 8% HAC reagent. Then, it was dissolved with DI water to make 250ppm mixture acid solution.

#### Sample preparation

Oranges were blended to make orange juice. 100ul of EGCG, P-EGCG, and EGCG-S tea solutions were added to 900ul orange juice to make samples as experimented groups, using 1ml original handmade orange juice as a control. 2.5ml of the acid reagent mixture was transferred to the samples. Then, the reagent was diluted to 25ppm.

#### Sample vitamin C detection

0.5ml, 1ml, 2.5ml, 5ml, and 12.5ml of 100ug/ml standard ascorbic acid solutions were diluted with DI water to 25ppm, using DI water as a blank control at 246 nm on the spectrophotometer to get UV – absorption data. The data was organized to make ascorbic acid standard calibration curve. The UV – absorption data for orange juice samples were tested at 25°C, 78°C for 0 day and 1 day respectively. Then, the data was analyzed to get vitamin C content based on ascorbic acid standard calibration curve.

#### **Statistics.**

All experiments were performed in triplicate. Data means  $\pm$  SD, and statistical analysis and liner regression were performed using SSPS version 23 for Apple OS.

Student's *t* tests or one-way ANOVA were used for comparing data. Generally,  $P < 0.05$  was considered significant in the study.

## RESULTS

### 1. EGCG, P-EGCG and EGCG-S in different conditions.

Both *L. burglarios*, and *S. thermophiles* were relatively resistant to homemade clementine orange juice (pH =3.03) in the control experiments. P-EGCG and EGCG-S inhibited more than 95% of *L. burglarios* growth during 30 minutes at 68°C in a water bath treatment before a one day 25°C incubation condition, while the percentage of the inhibition of EGCG was less than 50% for *L. burglarios*. CFU findings of *S. thermophiles* demonstrated that EGCG-S was more effective (85.10%) than P-EGCG (79.72%), and EGCG (39.68%) in inhibiting bacteria growth (Fig. 3A). For 30 minutes at 68°C in a water bath treatment after the one day 25°C incubated condition, all three green tea extractions only marginally restricted *S. thermophiles* bacteria growth (EGCG 19.40%, P-EGCG 44.57%, EGCG-S 29.94%). EGCG (-322.22%) was ineffective at reducing *L. burglarios* growth. Regardless of which bacteria were used in this environment, the percentage of inhibition of P-EGCG was better than that for EGCG, and EGCG-S (Fig. 3B). The differences among the three green tea extractions for 30 minutes of pasteurization before a room temperature incubation, were significant ( $P < 0.05$ ). There was no significant difference of bacteria inhibition of the three tea extractions for 30 minutes at a 68°C water bath treatment after a one day 25°C incubated condition period. (Table1). Pasteurization did not result in significant changes in tea polyphenols' stability.

## 2. Thermal inactivation at pasteurized temperature.

EGCG, P-EGCG, and EGCG-S inactivation were measured at two different temperatures conditions: 1) For 30- minutes at 68°C a water bath treatment (consistent with a low-temperature, long-time pasteurization) before a one day 25°C incubation period. All green tea extractions did inhibit bacteria growth in this condition (Table 1). 2) At 30- minutes 68°C water bath treatment (consistent with low-temperature, long-time pasteurization) after a one day 25°C incubated period. The EGCG was inactive in inhibiting *S. thermophiles* growth in this condition. Both P-EGCG and EGCG-S marginally constrained *L. burglarious*, and *S. thermophile* growth (Table 1). There was a significant difference ( $P < 0.05$ ) of the inhibition of *L. burglarious*, and *S. thermophiles* for 30-minute pasteurization and after a 24-hour room temperature incubation period. The percentage of inhibition of the two bacteria did not have significant differences ( $P > 0.05$ ) after 30-minute pasteurization before a room temperature incubation period.

**Table 1:** The inhibition of *L. burglarious* and *S. thermophiles* in calamine orange juice by three tea extractions at pasteurized temperature (68°C) for 30- minutes and 1 day. Values are the mean of three experiments.

Methods	Bacteria	green tea extractions	PH	% of inhibition	P value
pasteurized 30 min before room temperature treating	<i>L. burglarious</i>	EGCG		42.1	0.017
		P-EGCG		95.81	
		EGCG-S		99	
	<i>S. thermophiles</i>	EGCG		39.68	
		P-EGCG		79.72	
		EGCG-S	3.03	85.1	
pasteurized 30 min after room temperature treating	<i>L. burglarious</i>	EGCG		-322.22	0.386
		P-EGCG		66.67	
		EGCG-S		38.89	
	<i>S. thermophiles</i>	EGCG		19.4	
		P-EGCG		44.57	
		EGCG-S		29.94	

### 3. Ascorbic acid determination.

According to the the standard curve of ascorbic acid (AA) (see Supplemental File 3) and OD246 nm readings of clementine orange juice samples (Table 2), initial vitamin C contents of pure homemade juice were (387mg), EGCG (395mg), P-EGCG (405mg), and EGCG-S (406mg) /100 g of clementine orange juice respectively. After 15 seconds at the 78°C (consistent with high-temperature, short-time pasteurization) water bath process, the percentage decrease of AA contents of those samples were 0.517%, 7.34%, 0.74%, 4.43% respectively. In the second experimental condition, the samples were left in incubation at 25°C for 24 hours. The initial AA level of samples at room temperature were 372, 383, 403, 405mg/100g of juice respectively (Table 3). After storage for one day at 25°C and a 15-second 78°C water bath process, the percentage decrease of AA content were 5.11%,



5.74%, 0.992%, 4.69%, respectively (Table 4). At a 24-hour incubation period at 25°C, the percentage of decreasing of vitamin C in EGCG-S clementine orange juice concentrate had the lowest value compared to other concentrates (Table 4). Moreover, AA retentions in P-EGCG juice samples were found to be similar after a 24-hour incubation treatment and after pasteurization at 78°C in a water bath for 15 seconds and 24 hours at 25°C (Table 3). The percentage of AA was greater in juice with P-EGCG tea polyphenols than pure clementine orange juice after a 24-hour incubation period at 25°C (Table 4).

**Table 2:** The OD<sub>246</sub> nm data of the three clementine orange juice treatments at room temperature (25°C), and pasteurized temperature (78°C) for 0 day, 15 seconds and 1 day.

Values are the mean of three experiments.

	treatment methods	time	OD @ 246nm
orange juice	keep 25°C treatment, 24 hours	0 hour	0.471±0.006
EGCG			0.483±0.002
P-EGCG			0.495±0.001
EGCG-S			0.497±0.001
orange juice	78°C water bath 15 sec before 25°C treatment, 24 hours	15 secs	0.469±0.004
EGCG			0.442±0.003
P-EGCG			0.491±0.002
EGCG-S			0.472±0.001
orange juice	keep 25°C treatment, 24 hours	24 hours	0.451±0.002
EGCG			0.466±0.003
P-EGCG			0.494±0.005
EGCG-S			0.496±0.005
orange juice	78°C water bath 15 sec before 25°C treatment, 24 hours		0.424±0.003
EGCG			0.436±0.004
P-EGCG			0.487±0.004
EGCG-S			0.469±0.001
orange juice	78°C water bath 15 sec after 25°C treatment, 24 hours	1 day + 15secs	0.448±0.001
EGCG			0.451±0.002
P-EGCG			0.490±0.002
EGCG-S			0.493±0.001

**Table 3:** Ascorbic acid level at room temperature (25°C), and pasteurized temperature (78°C) for 0 day, 15 seconds and 1 day.

	time	pure orange juice		EGCG		P-EGCG		EGCG-S	
		AA (mg/100g)	SD	AA (mg/100g)	SD	AA (mg/100g)	SD	AA (mg/100g)	SD
Initial	0 hour	3.87	0.00 6	3.95	0.002	4.05	0.001	4.06	0.001
Study One	15 secs	3.85	0.00 4	3.66	0.003	4.02	0.002	3.88	0.001
	24 hours + 15sec	3.7	0.00 1	3.72	0.002	4.01	0.002	4.03	0.001
Study Two	24 hours a	3.72	0.00 2	3.83	0.003	4.03	0.005	4.05	0.005
	24 hours b	3.53	0.00 3	3.61	0.004	3.99	0.004	3.86	0.001

\*AA expressed as vitamin C concentration in orange juice. Values are the mean of three experiments.

\*SD Standard deviation.

\*a expressed as 25°C treating 24 hours

\*b expressed as 78°C water bath 15 sec after 25°C treating 24 hours

#### 4. Antioxidant capacity.

The antioxidant activity in clementine orange juice was measured by oxidant percentages for the three different green tea polyphenols (Table 4). The antioxidant ability in P-EGCG clementine orange juice was significantly higher than EGCG clementine orange juice in all selected temperature conditions (Table 5). The antioxidant capacity of clementine orange juice showed no significant correlations with the particular type of green tea polyphenol used ( $r = 0.528$ ,  $P > 0.05$ ). P-EGCG clementine orange juice showed the highest antioxidant capacity values as compared to the others (Table 4).

**Table 4:** The percentage decrease of ascorbic acid for 15 seconds and 1 day at room temperature (25°C), and pasteurized temperature (78°C).

methods	time	pure orange juice	EGCG	P-EGCG	EGCG-S
		decrease AA (%)	decrease AA (%)	decrease AA (%)	decrease AA (%)
pasteurized first	15 secs	0.517	7.34	0.74	4.43
pasteurized first	24 hours	3.9	-1.64	0.25	-3.87
room temperature	24 hours	3.88	3.03	0.49	0.25
pasteurized after	1 day + 15secs	5.11	5.74	0.99	4.69

**Table 5:** Antioxidant capacity of four orange juice types.

orange juice type	Antioxidant ability (%)
Pure	95.6050±0.0032ab
EGCG	93.8000±0.0028b
P-EGCG	99.0750±0.0028a
EGCG-S	97.4125±0.0018ab
P value	0.048

\*mean separate columns by Duncan's new multiple range test (p<0.05, n=4)

## DISCUSSION

Several studies showed that pH, and temperatures were important factors affecting the stability of EGCG (Balsaraf & Chole, 2015; Cui et al., 2012; Hirun & Roach, 2005; Labbé, Tremblay, & Bazinet, 2006; Widyaningrum et al., 2006). It has been reported that EGCG was unstable under higher- temperature environments (Hirun & Roach, 2005) and EGCG was found to be more stable at acidic pH than alkaline environments (Kumaran et al., 2008; Pollard et al., 2009; Zhong & Shahidi, 2012). The present study compared the stable ability of EGCG, and two more EGCG modifications (P-EGCG, EGCG-S) by using two different pasteurized methods. Even though AA did not impact the stability of EGCG

mixture products (Z.-Y. Chen et al., 1998), the data showed EGCG was unstable, and had a low impact on inhibiting bacteria growth for the two homemade clementine orange juice pasteurization experiments (pH =3.03). The results from the present study of EGCG are in agreement with the findings obtained in previous research (Cui et al., 2012; Hirun & Roach, 2005; Sakanaka et al., 1989). Both P-EGCG, and EGCG-S inhibited more bacteria growth than EGCG (Fig. 3A and 3B). This present study demonstrated that both P-EGCG and EGCG-S are considerably more resistant at higher temperatures and low pH values.

The findings of this investigation regarding vitamin C content also support the conclusions of other antioxidant studies (Hirun & Roach, 2005; H. Lee & Coates, 1999; Zhong & Shahidi, 2012). In a seminal study, Huang et al. (1997) found all EGCG derivatives could effectively inhibit oxidation in corn oil. Moreover, these derivatives have been found to have better or similar abilities to the parent normal EGCG molecule (Pollard et al., 2009). In the current study, the antioxidant activities of P- EGCG (99.075%), and EGCG-S (97.4125%) were very high compared to EGCG (93.8%). The findings supported previous findings that all three green tea polyphenols have strong antioxidant capabilities at room temperature. Moreover, P-EGCG and EGCG-S had a higher AOA than EGCG; building on research by Zhong & Shahidi (2012) that demonstrate higher temperatures had negative impact on AOA of EGCG. As expected, the vitamin C potency decreased with the increase of storage time of the citrus juice. The results showed P-EGCG and EGCG-S positively influenced the antioxidant capacity of clementine orange juice when subjected to temperature and time. Based on the AA level in Table 3, all green tea extractions increased the vitamin C values of clementine orange juice at room temperature. P-EGCG was a more effective polyphenol than EGCG or EGCG-S for increasing and protecting the

vitamin C values in the clementine orange juice. The results indicated that P-EGCG and EGCG-S are more stable than EGCG at lower pH values in a higher temperature environment. While all three green tea extractions can be considered as food additives in clementine orange juice, P-EGCG has the best potential for reduction of bacterial growth and maintenance of antioxidant properties during for clementine orange juice during storage. Therefore, P-EGCG could be considered as having the best potential among the tested polyphenols as a citrus juice preservative.

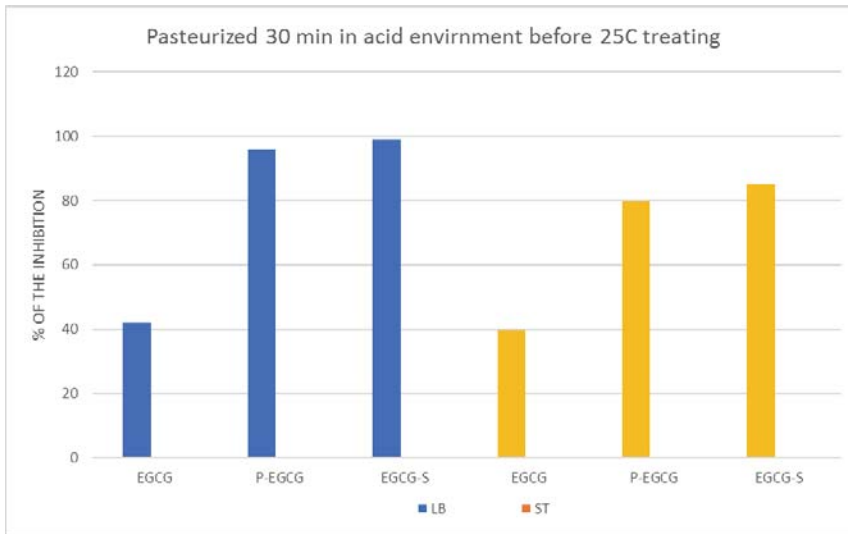
## **ACKNOWLEDGEMENTS**

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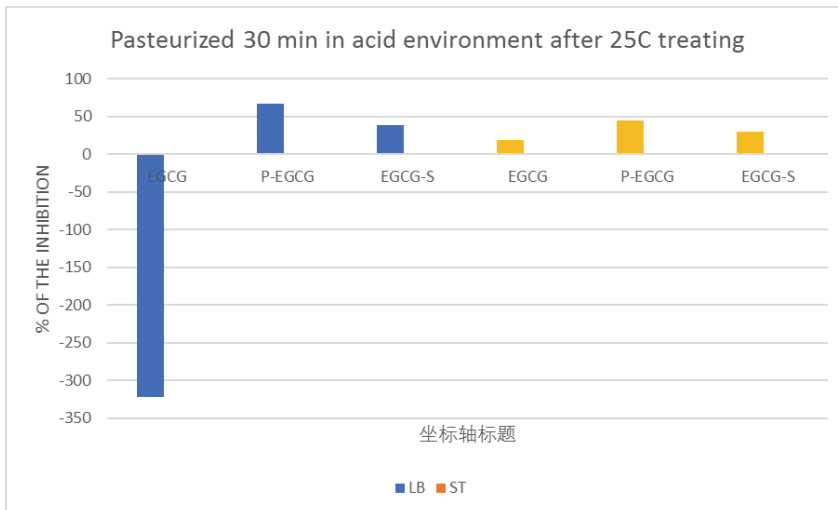
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Figure:**

**Figure 1A.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 68°C in 30-minutes before treatment of the bacteria in room temperature one day (n=3).

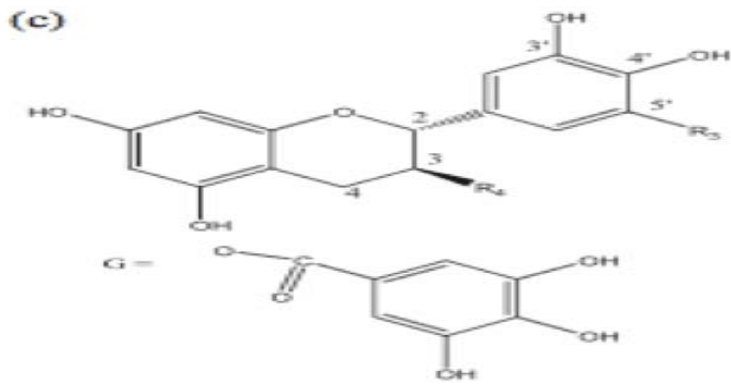


**Figure 1B.** The percentage of inhibition of EGCG, P-EGCG and EGCG-S at 68°C in 30 minutes after treatment with the bacteria in room temperature one day (n=3).

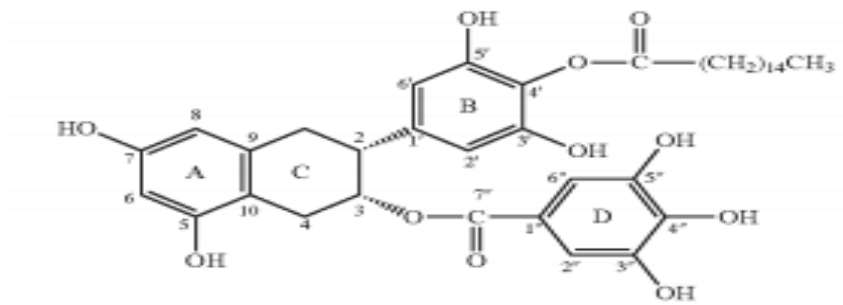


## Supplemental Files

1. Chemical structure of epigallocatechin-3-gallate (EGCG).

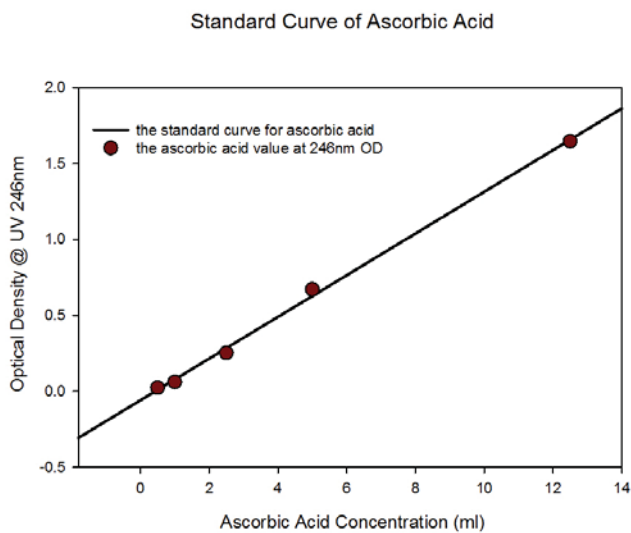


2. Chemical structure of EGCG-Sterate



3. The ascorbic acid standard curve in 25°C room condition.

\*Equation of the curve:  $Y = -0.0590 + (0.137 * X)$  ( $R = 0.999$   $R_{sq} = 0.998$ ,  $P < 0.001$ )





## **CONCLUSION AND IMPLICATIONS FOR STUDY #1 AND STUDY #2**

This thesis demonstrates that two green tea polyphenols (P-EGCG and EGCG-S) are stable at tested temperatures and pH conditions. Bacteria inhibition was the primary indicator of effectiveness, and P-EGCG showed a significantly higher rate of bacteria inhibition at temperatures and pH conditions used in these studies. Furthermore, all three of the green tea polyphenols did not degrade the antioxidant values of vitamin C in orange juice. In both studies P-EGCG was the best candidate to act as a potential food preservative in food industries.

Based on the statement of the limitations of these two manuscripts, it implies that future research should address the following issues:

Study the exact interactive biochemical mechanism(s) of P-EGCG that causes the inhibition of this variant of *E. coli*. Since the current study only considered inhibition of a single type of bacteria, further studies need to consider the effectiveness of P-EGCG with multiple types of bacteria present.

Explore the usability of green tea extractions for food manufacturing. A central question is whether EGCG, P-EGCG, and EGCG-S will negatively affect flavor of processed food. This is a valuable question to be considered, as it may affect marketability of products. Additionally, a long term stability study of green tea polyphenols is necessary for application in processed foods. For example, the bacterial inhibition properties at one month, six months, and one-year should be considered before adding tea polyphenols into food manufacturing processes. If P-EGCG, and EGCG-S are effective inhibitors of bacteria growth in longer time periods, manufacturers can offer customers a more socially acceptable natural preservative over chemical options.

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