



Green tea extract: a proposal for fresh vegetable sanitization

Rosiane Cosme NASCIMENTO¹, Jackline Freitas Brilhante de SÃO JOSÉ^{1,2*} 

Abstract

Sanitization is an essential step in reducing microorganisms since it ensures product safety for consumption. Medicinal plants have been widely used for such a purpose; among these is *Camellia sinensis* Linnaeus., which exerts antimicrobial action. However, studies on adopting this plant for sanitizing vegetables are scarce. The current study evaluates green tea's antimicrobial action against bacteria responsible for vegetable contamination. Green tea extracts (dry leaves and powder) at different concentrations were assessed based on the spectrum of action (1.0%, 2.5%, 5%, 7.5%, 10%), minimum inhibition concentration, and minimum bactericidal concentration (0.47%, 1.0%, 2.5%, 5%, 7.5%, 10%, 30%, 60%). A challenge test was applied to tomatoes intentionally contaminated with pathogens to evaluate green tea extracts (30% and 60%). Application of 10% green tea extract impeded the formation of inhibition zones *Escherichia coli*, *Salmonella enterica* serovar Enteritidis (*S. Enteritidis*), *Staphylococcus aureus*, and *Enterococcus faecalis* in 7.41 mm, 6.83 mm, 7.33 mm, and 6.58 mm, respectively. The count of *S. Enteritidis* adhered to surfaces of the tomatoes decreased most significantly after treatment application. Thus, based on the current results, green tea leaf extract exhibits antimicrobial action, which makes it a potential natural sanitizer.

Keywords: sanitizers; *Camellia sinensis* L.; quality; antibacterial activity; fresh vegetables.

Practical Application: Antimicrobial potential of aqueous *Camellia sinensis* L. extract for sanitization of vegetables.

1 Introduction

Minimal processing of fruits and vegetables involves washing, peeling, cutting, sanitizing, centrifuging, and packaging them for their immediate consumption or preparation (Rodgers, 2016; Alenyorege et al., 2020). Ready-to-eat products cause consumer concern about the microbiological quality of food. In addition, foodborne diseases have been associated with consumption of fruit and vegetables contaminated with pathogenic viruses, parasites, or bacteria (Bhilwadikar et al., 2019). Accordingly, washing and sanitizing fruits and vegetables are crucial steps to achieve high-quality microbiological effects. Therefore, it is essential to choose an effective microbial sanitizer that does not pose risks to consumers or the environment (São José, 2017; Cossu et al., 2017; Alvarenga et al., 2020; Pelissari et al., 2021).

Most sanitizers used in the food industry are based on chlorine or chlorinated compounds, mainly used on fresh products (São José et al., 2014; Duarte et al., 2018; Ortiz-Solà et al., 2020); however, the efficiency of chlorine in reducing pathogen contamination has been questioned. In addition, high chlorination of wastewater is likely associated with high organic carbon content and can increase levels of trihalomethanes and other disinfection byproducts (Rosário et al., 2017; Duarte et al., 2018; São José et al., 2018; Ortiz-Solà et al., 2020). According to Lepaus et al. (2020) and Pelissari et al. (2021), these compounds are highly carcinogenic and could cause environmental problems. Furthermore, studies have indicated that long-term exposure to disinfection byproducts, such as trihalomethanes, is related to higher incidence of harmful health consequences, such as bladder cancer (Li et al., 2021). Thus, it is essential to search

for new antimicrobial compounds, such as *Camellia sinensis* L. green tea, as alternatives to chemical sanitizers (Randazzo et al., 2017; Verrillo et al., 2021; Kang & Song, 2021).

Camellia sinensis L. belongs to the family Theaceae and is popularly known as green tea or Indian tea. Green tea is used as a medicinal herb, since this plant contains flavonoids and catechins with powerful therapeutic activities, such as antioxidant, metal chelation, and lipoperoxidation inhibition (Zhang et al., 2021). This extract has also been used in active food packaging to improve product quality and safety for consumers and extend packaged food shelf life (Carrizo et al. 2016; Lorenzo & Munekata, 2016; Martins, 2018). Green tea's therapeutic properties are mainly attributed to polyphenolic compounds, including epicatechin, epigallocatechin, flavonoids, and caffeine (Liu et al., 2016; Chen et al., 2016). Furthermore, *Camellia sinensis* L. green tea extract has been described to have antimicrobial effects against bacteria, fungi, and viruses (Reygaert, 2014; Marti et al., 2017) related to polyphenols (Fernández et al., 2018). In addition, catechins are compounds demonstrated to have antibacterial effects (Marti et al., 2017). Some studies carried out *in vitro* and *in vivo* have shown the anticarcinogenic and antimicrobial action of green tea polyphenols in promoting the inhibition and inactivation of *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Chacko et al., 2010).

These pathogens are involved in outbreaks of foodborne illness, a global health problem, which makes prevention extremely important. The most common microorganisms involved in this

Received 28 Jul., 2021

Accepted 16 Apr., 2022

¹Programa de Pós-graduação em Nutrição e Saúde, Universidade Federal do Espírito Santo, Vitória, ES, Brasil

²Departamento de Educação Integrada em Saúde, Centro de Ciências da Saúde, Universidade Federal do Espírito Santo, Vitória, ES, Brasil

*Corresponding author: jackline.jose@ufes.br

problem include *Salmonella*, Norovirus, *Staphylococcus aureus*, *Shigella*, *Campylobacter*, *Clostridium botulinum*, pathogenic *Escherichia coli* O157:H7 and O104:H4, *Listeria* spp., and *Vibrio* spp. (Bhilwadikar et al., 2019). There are many possibilities for contamination of fresh produce in the preharvest phases through contaminated seeds, irrigation water, soil, and pests and in postharvest processes such as contamination on equipment surfaces, cross-contamination from cutting boards, transport, or inappropriate handling (Bhilwadikar et al., 2019).

Reports in the scientific literature have pointed out the antimicrobial action of green tea extract in inhibiting *Escherichia coli* and *Staphylococcus aureus* (Zihadi et al., 2019), enteric viruses (Randazzo et al., 2017; Falcó et al., 2018, 2020), and *Bacillus cereus* (Patil et al., 2016). However, there are few reports regarding its antimicrobial action against bacteria account for its usefulness in reducing fruit and vegetable contamination. Therefore, this study aimed to evaluate the antimicrobial action of different concentrations of green tea (*Camellia sinensis* L.) on bacteria responsible for vegetable contamination.

2 Materials and methods

2.1 Green tea acquisition and preparation of sanitizing solution

Green tea (*Camellia sinensis* L. var. *assamica*) was purchased in a local grocery shop in the city of Vitória, Espírito Santo State, Brazil, in the forms of dry leaves (Yamamoto®, Pinheiro, São Paulo, Brazil) and extract powder (Santos Flora Comércio de Ervas®, Mairiporã, São Paulo, Brazil). These products were stored in containers protected from light at 8 °C until analysis. Aqueous extracts were prepared of dried green tea leaf at concentrations of 0.47%, 1%, 2.5%, 5%, 7.5%, 10%, 30% and 60% through infusion in water for 5 min. Each sample was filtered under aseptic conditions after the extraction process. The same green tea concentrations were used to prepare powdered tea extracts under the same conditions adopted for leaf extracts.

2.2 Analysis of the antimicrobial action of green tea extracts

Bacteria responsible for vegetable contamination were assessed to investigate the spectrum of action, the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC), and the effects at the tested green tea extract concentrations. In addition, antimicrobial action was tested against four bacterial strains divided into Gram-positive (*Staphylococcus aureus* ATCC 6538 and *Enterococcus faecalis* ATCC 51299) and Gram-negative (*Escherichia coli* ATCC 11229 and *Salmonella enterica* serovar Enteritidis ATCC 13076) groups. These cultures were obtained from the culture stock of the Food Hygiene and Microbiology Laboratory of the Federal University of Viçosa and stored at -80 °C in brain heart infusion (BHI) agar (Himedia®, India) and glycerol (80:20). Bacteria used in the experiment were activated twice in tubes filled with BHI broth (Himedia®, India) and incubated at 37 °C for 18 h.

Agar diffusion test

The spectrum of antimicrobial action was determined by inhibiting microbial multiplication through diffusion in agar, based

on the methodology described by the Clinical and Laboratory Standards Institute (2003), with modifications. Mueller-Hinton (Himedia®, India) agar was prepared, and after sterilization, 25 mL of it was distributed in Petri dishes to ensure a uniform depth of approximately 4 mm. After drying, each Mueller-Hinton agar plate was inoculated with a swab dipped in bacterial solution (1.5×10^8 CFU/mL) with turbidity equivalent to 0.5 McFarland standard (Probac, Brazil). A hole (5 mm in diameter) was made in the center of each inoculated plate. Subsequently, 30 µL of each green tea extract was separately placed into an agar cavity. The plates were kept under refrigeration at a temperature ranging from 7 °C to 10 °C overnight for antimicrobial diffusion and then incubated at 37 °C for 18 h. Then, the inhibition zones were measured in millimeters. The magnitude of the diameter of an inhibition zone was assumed to reflect the antimicrobial activity of the extracts. For this step, the extract concentrations applied were 1%, 2.5%, 5%, 7.5%, and 10%.

Minimum inhibitory concentration and minimum bactericidal tests

In this step, the extract concentrations applied were 0.47%, 1%, 2.5%, 5%, 7.5%, 10%, 30% and 60%. MIC values were determined by the macrodilution tube method based on the guidelines of the Clinical and Laboratory Standards Institute (2003). Tubes with extracts were inoculated with approximately 5.0×10^5 CFU/mL of each bacterium and incubated at 37 °C for 18 h. Positive tubes were those exhibiting visible turbidity, and the negative tubes did not show visible turbidity. The MIC is the lowest antimicrobial concentration inhibiting visible bacterial growth.

Tubes classified as negative (without visible growth) were analyzed for MBC determination. All liquid was transferred to previously autoclaved 15 mL tubes after homogenization in a vortex agitator (Phoenix-Lufesco® AP59, Araraquara, São Paulo, Brazil). Each sample was centrifuged at 4,000 x g (Kasvi® K14-0815^a, São José dos Pinhais, Paraná, Brazil) for 5 min, and the supernatants were discarded. Cells were washed twice in 10 mL of 0.85% m/v saline solution to remove the antimicrobial agents. Each pellet obtained was resuspended in 100 µL of saline solution; the entire volume was inoculated in Petri dishes filled with standard agar for PCA counting (Himedia®, India). The plates were incubated at 37 °C for 24 h. MBC was the lowest concentration causing total inhibition of microbial growth after inoculation in Petri dishes. The negative control consisted of a broth medium without inoculum and green tea extract. The positive growth control contained pure microbial culture without an antimicrobial agent.

2.3 Challenge test applied to tomato intentionally contaminated with pathogens

The two best green tea extract solutions (30% and 60%) based on the minimum inhibitory concentration and minimum bactericidal tests as described in Section 2.2 were chosen for challenge tests. This analysis was carried out according to the method described by São José et al. (2014), with slight adaptations.

Tomato samples were purchased in a local grocery shop in Vitória City, Espírito Santo State, Brazil, and were selected and

washed in running water for dirt removal. The samples were placed in sterile plastic bags filled with active suspensions (10^6 CFU/mL) of each culture. The suspensions were drained after one hour at 25 °C; the tomatoes were then incubated at 25 °C for 24 h. After incubation, pieces (1.0 x 1.0 cm) were aseptically cut from the tomatoes with the aid of a sterile scalpel and placed in sterilized Petri dishes until the sanitization time. The cuts were made so that only the tomatoes' outer surfaces were removed. Pieces with adhered cells were maintained statically for 1 min in 10 mL of 0.1% peptone water to remove planktonic cells. Subsequently, tomato pieces were immersed in 10 mL of this same solution and stirred in a vortex tube agitator for 1 min for sessile cell removal. Appropriate dilutions were made and inoculated in Petri dishes filled with brain heart infusion agar (Himedia®, India). The samples were then incubated at 37 °C for 24 h. The number of adhered cells (number of initial cells before sanitization) was expressed in CFU/cm² (São José et al., 2014). Tomato pieces were immersed in 10 mL of the two solutions with green tea for 5 min after planktonic cell removal. Then, they were immersed in 10 mL of sterilized distilled water for 1 min to remove sanitizing solution residues. Tomato pieces were then immersed in 10 mL of 0.1% peptone water and subjected to homogenization in a vortex stirrer® for 1 min to remove the surviving cells. Appropriate dilutions were made and inoculated in Petri dishes filled with brain heart infusion agar; the samples were then incubated at 37 °C for 24 h. The number of surviving cells (number of final cells - after sanitization) was expressed in CFU/cm² (São José et al. 2014).

2.4 Statistical analysis

The experiment followed a completely randomized design, with three repetitions. Data on inhibition zone sizes and mean CFU/cm² values before and after sanitization were subjected to analysis of variance (ANOVA) and compared through Duncan's tests at a 5% probability level. SAS software, online version (SAS Institute Inc., Cary, NC, USA), was used for the statistical analysis.

3 Results and discussion

3.1 In vitro analysis of the antimicrobial action of green tea extracts

E. coli and *S. aureus* showed more satisfactory results of microbial inhibition by aqueous green tea leaf extract at the tested concentrations than did *Salmonella* Enteritidis and *Enterococcus faecalis* (Table 1).

When comparing the lowest and highest concentrations used, the application of 10% aqueous green tea leaf extract caused increases of 2.25 mm, 1.83 mm, 2.30 mm, and 1.17 mm in the inhibition zone diameters surrounding colonies of *E. coli*, *Salmonella* Enteritidis, *S. aureus*, and *Enterococcus faecalis*, respectively (Table 1). According to Fernández et al. (2018), green tea extract exerts antibacterial activity against Gram-positive as well as Gram-negative bacteria. In this research, when green tea leaf extract was used, there were differences in the mean diameters of the zones of inhibition ($p < 0.05$) of *E. coli* and *S. aureus* between the 1.0% extract and more concentrated extracts. Similar results were found by Alvarenga et al. (2007), who tested Gram-positive microorganisms (*S. aureus*, *L. monocytogenes*, *S. mitis*, and *S. mutans*) that showed resistance to plant extracts. However, they used concentrations of 20% and found larger inhibition zones than those recorded in our study. Epigallocatechin gallate, present in green tea, causes different effects on Gram-positive and Gram-negative bacteria. In Gram-positive bacteria, this compound binds to peptidoglycans and causes their precipitation and damage to the cell wall and, therefore, modifies biosynthesis. In Gram-negative bacteria, this effect also occurs, but damage is induced mainly by H₂O₂ production with impacts on membrane fluidity and pore-like lesions (Fernández et al., 2018).

The aqueous green tea leaf extract concentration showed antibacterial effects at the lowest concentration (10%) in comparison to results of the study by Alvarenga et al. (2007). However, according to Hamilton-Miller (1995), factors not related to extraction concentration can also influence inhibition zone size, for instance, the number of polyphenolic compounds, which are essential for green tea's antimicrobial action. Furthermore, external factors, such as climate, growing location, and weather conditions, can influence secondary metabolite content in plants and the antibacterial action of the extract (Arruda et al., 2021). Irineu & Borges (2014) showed that an increase in green tea concentration directly influences the inhibition zone size, finding that *Streptococcus pneumoniae* was more sensitive to this extract at higher *Camellia sinensis* L. concentrations. That is, the inhibitory effect occurs in a dose-dependent manner.

The 10% green tea powder extract application promoted increases in inhibition zones of 6.67 mm, 5.33 mm, 5.67 mm, and 5.99 mm for *E. coli*, *Salmonella* Enteritidis, and *S. aureus*, respectively (Table 2). The higher the extract concentration was, the larger the inhibition zone; however, according to established international standards (Clinical & Laboratory Standards Institute, 2003), the microorganisms tested were more resistant

Table 1. Mean diameter (mm) of microbial inhibition zones for aqueous green tea leaf extract.

Concentration	Mean diameter (mm)			
	<i>E. coli</i>	<i>Salmonella</i> Enteritidis	<i>S. aureus</i>	<i>Enterococcus</i>
1.0%	5.16 ^c ± 1.04	5.00 ^a ± 0.50	5.03 ^b ± 0.05	5.41 ^a ± 0.38
2.5%	5.58 ^{bc} ± 0.52	5.50 ^a ± 0.86	5.91 ^{ab} ± 0.80	5.66 ^a ± 0.80
5.0%	6.58 ^{ab} ± 0.38	6.08 ^a ± 1.01	6.25 ^{ab} ± 1.10	5.83 ^a ± 0.76
7.5%	6.91 ^a ± 0.38	6.50 ^a ± 0.90	7.08 ^a ± 0.95	6.33 ^a ± 1.15
10.0%	7.41 ^a ± 0.52	6.83 ^a ± 1.18	7.33 ^a ± 0.76	6.58 ^a ± 0.94

All experiments were conducted in triplicate. Means followed by the same letter in the same column did not differ by Duncan's test ($p < 0.05$).

Table 2. Mean of microbial inhibition zone diameter (mm) for aqueous green tea powder extract action.

Concentration	Mean* diameter (mm)			
	<i>E. coli</i>	<i>Salmonella</i> Enteritidis	<i>S. aureus</i>	<i>Enterococcus</i>
1.0%	5.66 ^c ± 0.57	6.00 ^c ± 1.00	6.33 ^c ± 0.57	6.01 ^d ± 0.01
2.5%	6.50 ^{bc} ± 0.86	7.00 ^{bc} ± 2.08	7.50 ^{bc} ± 2.17	7.33 ^c ± 0.58
5.0%	8.83 ^{ab} ± 1.25	8.33 ^{abc} ± 0.57	9.00 ^{bc} ± 1.01	8.34 ^{bc} ± 0.57
7.5%	9.33 ^{ab} ± 0.57	9.66 ^{ab} ± 2.30	10.33 ^{ab} ± 2.30	9.33 ^b ± 0.50
10.0%	12.33 ^a ± 2.30	11.33 ^a ± 1.52	12.00 ^a ± 2.64	12.00 ^a ± 0.02

All experiments were conducted in triplicate. *Means followed by the same letter in the same column did not differ from each other by Duncan's test ($p < 0.05$).

since the inhibition zones that formed around them did not exceed 15 mm. Wong-Leung (1988) proposed that inhibition zone formation equal to or greater than 10 mm is indicative of a compound's antimicrobial action. Considering this reference, the 10% concentration showed efficient antimicrobial action in the four tested bacterial strains. In addition, the 7.5% extract concentration showed antimicrobial action in *S. aureus*.

The inhibition zone size can be influenced by the diffusion capacity of each type of antimicrobial agent. The CLSI standardized method was developed to evaluate conventional antimicrobial agents such as antibiotics. Overall, such substances are hydrophilic and quickly diffuse in the agar. However, volatile substances that are insoluble in water or that have more complex chemical composition, such as plant essential oils, can have lower diffusion ability (Montanari et al., 2012).

Camellia sinensis L. extract showed a bacteriostatic effect on all assessed microorganisms (Table 3). Tests were performed, and the MIC was equal to 30% for *E. coli* and *S. aureus* and 60% for *S. Enteritidis* and *Enterococcus*. None of the assessed concentrations allowed bacterial inactivation; this finding indicates a bacteriostatic effect.

Michelin et al. (2005) analyzed activity of extracts of several plant species, such as *Artemisia absinthium* (wormwood), *Mentha pulegium* (pennyroyal), *Punica granatum* (pomegranate), *Xanthosoma sagittifolium* (taioba), and *Syzygium cumini* (jambolan), at a concentration of 200 mg/mL against microorganisms such as *S. aureus* and *Candida albicans*. Researchers have observed the inhibitory effect of extracts on bacteria, underscoring the importance of the antimicrobial action of natural compounds (Farooqui et al., 2015). Özvural et al. (2016) evaluated the microbiological characteristics of hamburgers coated with edible green tea extract compared to a control sample with a chitosan coating. They found lower mesophilic aerobic count values in samples coated with green tea extract. This result demonstrated the antimicrobial effect of green tea on food. Although this research did not carry out the sensory analysis of foods treated with green tea extract, this evaluation should be emphasized. Green tea extract is composed of catechins, consequently providing bitterness and astringency (Çakmakçi et al., 2019).

It is essential to highlight that, based on MIC results, the best treatment was chosen for the challenge test applied to the vegetable samples. Green tea leaf extract was adopted because green tea is easier to find in the market. Studies have shown that the biological and pharmacological effects of green tea extract (anti-inflammatory, antimicrobial, antitumor, and antioxidant)

Table 3. Values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of green tea extracts in different bacteria.

Bacterial species	Green tea extract (%)	
	MIC	MBC
<i>E. coli</i>	30	>30
<i>S. Enteritidis</i>	60	>60
<i>S. aureus</i>	30	>30
<i>Enterococcus faecalis</i>	60	>60

All experiments were conducted in triplicate. The MIC was defined as the lowest extract concentration that fully inhibited microbial growth after 18 h of incubation at 37 °C in Mueller-Hinton broth.

result from catechins found in plant leaves. In addition, all catechins have synergistic effects, so green tea leaf extract accounts for more significant antimicrobial action than do the catechins isolated from it (Wanasundara & Shahidi, 1998; Sharma et al., 2012).

3.2 Challenge test applied to vegetables intentionally contaminated with pathogens

There was a significant difference in microorganism count between non-sanitized tomatoes and tomatoes sanitized with green tea ($p < 0.05$) (Table 4). The application of different concentrations of green tea extracts resulted in reductions of 1.19 to 7.03 log CFU/cm² in the different microorganisms evaluated. According to São José & Vanetti (2015), the efficiency of chlorinated compounds in reducing microbial contamination in vegetables is limited; it reaches one to two of the logarithmic cycles of microorganisms. Therefore, the current results were consistent with the reductions commonly found in treatments with chlorine-based compounds. The sanitization time result recorded herein was outstanding; it was equal to 5 min contact.

After treatment of *E. coli* and *S. aureus* with 60% green tea extract, reductions at a level that was "not determined" indicated the greatest reductions (Table 4). On the other hand, *E. coli* and *S. aureus* showed significant reductions compared to non-sanitized controls when 30% green tea extract was applied, with reductions of 1.19 and 1.47 log CFU/cm², respectively ($p < 0.05$).

According to Rocha et al. (2010), washing and sanitizing fruits and vegetables prevents diseases caused by foodborne pathogens. Most sanitizers used by the food industry and food services are based on chlorine and chlorinated compounds (Chen & Zhu, 2011). Low cost, high antimicrobial action, product practicality, easy handling, and complete dissolution in water are the main factors influencing the use of chlorinated compounds as food

Table 4. Effects of different green tea extract concentrations applied for 5 min to reduce bacteria count intentionally adhered to tomato surfaces (*Solanum lycopersicum* L.).

Green Tea Extract	Log CFU/cm ²			
	<i>E. coli</i>	<i>Salmonella</i> Enteritidis	<i>S. aureus</i>	<i>Enterococcus</i>
0%	6.33 ^a ± 0.06	6.81 ^a ± 0.59	7.03 ^a ± 0.39	6.60 ^a ± 0.32
30%	5.14 ^b ± 0.14	n.d.	5.56 ^b ± 0.69	n.d.
60%	n.d.	5.23 ^b ± 0.14	n.d.	5.32 ^b ± 0.07

All experiments were conducted in triplicate. Means followed by the same letter in the same column did not differ by Duncan's test ($p < 0.05$). n.d. - not determined at the lowest prepared dilution.

disinfectants (São José & Vanetti, 2015). However, Oliveira et al. (2012) found that 88% of visited commercial restaurants did not use sanitizers at the correct concentrations set by legislation or at the expected action time (15 minutes), as recommended. The appropriate sanitizer concentration is essential; therefore, attention must be paid at the time it is applied, since high concentrations can cause sensory changes in food and make it unacceptable (Lepaus et al., 2020).

Chlorinated compounds such as sanitizers are questioned, given the likely occurrence of wastewater hyperchlorination (São José et al., 2014; Rosário et al., 2017; São José et al., 2018; Alvarenga et al., 2020). Time and excessive amounts of chlorinated compounds are associated with high organic carbon content, and this process results in high concentrations of trihalomethanes, which are considered carcinogenic substances (Marti et al., 2017). *Camellia sinensis* L. is rich in polyphenols, such as catechins, which account for antioxidant, anti-inflammatory, antifungal, and antimicrobial action (Bernegossi et al. 2016; Camargo et al. 2016). Some antimicrobial mechanisms of green tea include bacterial cell membrane destruction, enzyme activity inhibition, and inhibition of fatty acid synthesis (Reygaert, 2014).

Some considerations are essential to make possible the application of green tea extract. One is that the efficacy of green tea extract is directly related to tea polyphenol content. Another is related to the composition and characteristics of the food to which it is applied. According to Fernández et al. (2018), it is necessary to understand the interactions between antimicrobials and food components.

Based on the results, green tea can be applied as a vegetable sanitizer because it was able to reduce microbial counts at levels similar to or better than those of chlorinated compounds. Furthermore, the use of natural sanitizers is a trend in the context of concern about the environmental impacts of chemical products and meets a conscious consumer demand that seeks to consume foods that are processed in a more sustainable manner. Thus, *Camellia sinensis* L. extract can contribute to decontamination of vegetables and reduce the time that sanitizers are in contact with food.

4 Conclusion

Green tea extract exerts antimicrobial activity against *Escherichia coli*, *Salmonella* Enteritidis, *Staphylococcus aureus*,

and *Enterococcus faecalis*, and the highest antibacterial effects were obtained with 10% extracts. The MIC was equal to 30% in *E. coli* and *S. aureus* and 60% in *S. Enteritidis* and *Enterococcus*. The count of *Salmonella* Enteritidis adhered to tomato surfaces decreased the most after treatment application compared to the other strains. Thus, based on the current results, green tea leaf extract has antimicrobial action, which makes it a potential natural sanitizer that could replace chlorine compounds. However, further studies are needed to evaluate other green tea extract concentrations and contact times to assess its antimicrobial effect in vegetable sanitation. In addition, one should seek to understand the implications of this natural compound for bacterial cells and to assess sensory food quality after treatment application.

Acknowledgements

The authors are grateful to Espírito Santo State Research and Innovation Support Foundation (FAPES) for the financial support granted to project number 554/2015, approved by Edital Fapes n. 006/2014. We thank the Research Support Fund of the Federal University of Espírito Santo (FAP/UFES) for all the support. Furthermore, the authors are grateful to Coordination for the Improvement of Higher Education Personnel (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES*) for supporting the Graduate Program in Nutrition and Health.

References

- Alenyorege, E. A., Ma, H., Aheto, J. H., Ayim, I., Chikari, F., Osa, R., & Zhou, C. (2020). Response surface methodology centred optimization of mono-frequency ultrasound reduction of bacteria in fresh-cut Chinese cabbage and its effect on quality. *LWT- Food Science and Technology*, 122, 108991. <https://doi.org/10.1016/j.lwt.2019.108991>.
- Alvarenga, A. L., Schwan, R. F., Dias, D. R., Schulan, K. R. F., & Bravo, C. E. C. (2007). Atividade antimicrobiana dos extratos vegetais sobre bactérias patogênicas humanas. *Revista Brasileira de Medicina*, 9(4), 86-89.
- Alvarenga, P. D. L., Vasconcelos, C. M., & São José, J. F. B. (2020). Application of ultrasound combined with acetic acid and peracetic acid: microbiological and physicochemical quality of strawberries. *Molecules (Basel, Switzerland)*, 26(1), 1-16. PMID:33375142.
- Arruda, T. R., Pinheiro, P. F., Silva, P. I., & Bernardes, P. C. (2021). A new perspective of a well-recognized raw material: phenolic content, antioxidant and antimicrobial activities and α - and β -acids profile of Brazilian hop (*Humulus lupulus* L.) extracts. *Lebensmittel-Wissenschaft + Technologie*, 141, 110905. <http://dx.doi.org/10.1016/j.lwt.2021.110905>.
- Bernegossi, J., Barbosa, R. M. C., Rustice, P. M., & Chorilli, M. (2016). Green tea glycolic extract-loaded liquid crystal systems: development, characterization and microbiological control. *Brazilian Journal of Pharmaceutical Sciences*, 52(3), 383-390. <http://dx.doi.org/10.1590/s1984-82502016000300004>.
- Bhilwadikar, T., Pounraj, S., Manivannan, S., Rastogi, N. K., & Negi, P. S. (2019). Decontamination of Microorganisms and Pesticides from Fresh Fruits and Vegetables: A Comprehensive Review from Common Household Processes to Modern Techniques. *Comprehensive Reviews in Food Science and Food Safety*, 18(4), 1003-1038. <http://dx.doi.org/10.1111/1541-4337.12453>. PMID:33337007.
- Camargo, L. E. A., Pedroso, L. S., Vendrame, S. C., Mainardes, R. M., & Khalil, N. M. (2016). Antioxidant and antifungal activities of

- Camellia sinensis* (L.) Kuntze leaves obtained by different forms of production. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, 76(2), 428-434. <http://dx.doi.org/10.1590/1519-6984.18814.PMid:26983085>.
- Carrizo, D., Tabora, G., Nérin, C., & Bosetti, O. (2016). Extension of shelf life of two fatty foods using a new antioxidant multilayer packaging containing green tea extract. *Innovative Food Science & Emerging Technologies*, 33, 534-541. <http://dx.doi.org/10.1016/j.ifset.2015.10.018>.
- Chacko, S. M., Thambi, P. T., Kuttan, R., & Nishigaki, I. (2010). Beneficial effects of green tea: a literature review. *Chinese Medicine*, 5, 13. <http://dx.doi.org/10.1186/1749-8546-5-13>. PMID:20370896.
- Chen, I. J., Liu, C. Y., Chiu, J. P., & Hsu, C. H. (2016). Therapeutic effect of high-dose green tea extract on weight reduction: a randomized, double-blind, placebo-controlled clinical trial. *Clinical Nutrition (Edinburgh, Scotland)*, 35(3), 592-599. <http://dx.doi.org/10.1016/j.clnu.2015.05.003>. PMID:26093535.
- Chen, Z., & Zhu, C. (2011). Combined effects of aqueous chlorine dioxide and ultrasonic treatments on postharvest storage quality of plum fruit (*Prunus salicina* L.). *Postharvest Biology and Technology*, 61(2-3), 117-123. <http://dx.doi.org/10.1016/j.postharvbio.2011.03.006>.
- Clinical & Laboratory Standards Institute – CLSI. (2003). *Padronização dos testes de sensibilidade a antimicrobianos: norma aprovada* (vol. 23, no. 1). Wayne: NCCLS/CLSI.
- Cossu, A., Le, P., Young, G. M., & Nitin, N. (2017). Assessment of sanitation efficacy against *Escherichia coli* O157:H7 by rapid measurement of intracellular oxidative stress, membrane damage or glucose active uptake. *Food Control*, 71, 293-300. <http://dx.doi.org/10.1016/j.foodcont.2016.07.009>.
- Duarte, A. L. A., do Rosário, D. K. A., Oliveira, S. B. S., de Souza, H. L. S., de Carvalho, R. V., Carneiro, J. C. S., Silva, P. I., & Bernardes, P. C. (2018). Ultrasound improves antimicrobial effect of sodium dichloroisocyanurate to reduce *Salmonella* Typhimurium on purple cabbage. *International Journal of Food Microbiology*, 269, 12-18. <http://dx.doi.org/10.1016/j.ijfoodmicro.2018.01.007>. PMID:29353020.
- Falcó, I., Randazzo, W., Gómez-Mascaraque, L., Aznar, R., López-Rubio, A., & Sánchez, G. (2018). Fostering the antiviral activity of green tea extract for sanitizing purposes through controlled storage conditions. *Food Control*, 84, 485-492. <http://dx.doi.org/10.1016/j.foodcont.2017.08.037>.
- Falcó, I., Díaz-Reolid, A., Randazzo, W., & Sánchez, G. (2020). Green tea extract assisted low-temperature pasteurization to inactivate enteric viruses in juices. *International Journal of Food Microbiology*, 334, 108809. <https://doi.org/10.1016/j.ijfoodmicro.2020.108809>.
- Farooqui, A., Khan, A., Borghetto, I., Kazmi, S. U., Rubino, S., & Paglietti, B. (2015). Synergistic antimicrobial activity of *Camellia sinensis* and *Juglans regia* against multidrug-resistant bacteria. *PLoS One*, 10(2), e0118431. <http://dx.doi.org/10.1371/journal.pone.0118431>. PMID:25719410.
- Fernández, M. V., Agüero, M. V., & Jagus, R. J. (2018). Green tea extract: a natural antimicrobial with great potential for controlling native microbiota, *Listeria innocua* and *Escherichia coli* in fresh-cut beet leaves. *Journal of Food Safety*, 38(1), 1-9. <http://dx.doi.org/10.1111/jfs.12374>.
- Hamilton-Miller, J. M. (1995). Antimicrobial properties of tea (*Camellia sinensis* L.). *Antimicrobial Agents and Chemotherapy*, 39(11), 2375-2377. <http://dx.doi.org/10.1128/AAC.39.11.2375>. PMID:8585711.
- Irineu, L. E. S. S., & Borges, K. C. A. S. (2014). Efeito antibacteriano de *Camellia sinensis* sobre patógenos humanos. *Cadernos UniFOA*, 24, 65-69.
- Kang, J., & Song, K. B. (2021). Antimicrobial activity of honeybush (*Cyclopia intermedia*) ethanol extract against foodborne pathogens and its application in washing fresh-cut Swiss chard. *Food Control*, 121, 107674. <http://dx.doi.org/10.1016/j.foodcont.2020.107674>.
- Lepaus, B. M., Rocha, J. S., & São José, J. F. B. (2020). Organic acids and hydrogen peroxide can replace chlorinated compounds as sanitizers on strawberries, cucumbers and rocket leaves. *Food Science and Technology (Campinas)*, 40(suppl 1), 242-249. <http://dx.doi.org/10.1590/fst.09519>.
- Li, R. A., McDonald, J. A., Sathasivan, A., & Khan, S. J. (2021). A multivariate Bayesian network analysis of water quality factors influencing trihalomethanes formation in drinking water distribution systems. *Water Research*, 190, 116712. <https://doi.org/10.1016/j.watres.2020.116712>.
- Liu, Z., Chen, Z., Guo, H., He, D., Zhao, H., Wang, Z., Zhang, W., Liao, L., Zhang, C., & Ni, L. (2016). The modulatory effect of infusions of green tea, oolong tea, and black tea on gut microbiota in high-fat-induced obese mice. *Food & Function*, 7(12), 4869-4879. <http://dx.doi.org/10.1039/C6FO01439A>. PMID:27812583.
- Lorenzo, J. M., & Munekata, P. E. S. (2016). Phenolic compounds of green tea: health benefits and technological application in food. *Asian Pacific Journal of Tropical Biomedicine*, 6(8), 709-719. <http://dx.doi.org/10.1016/j.apjtb.2016.06.010>.
- Marti, E., Ferrary-Américo, M., & Barardi, C. R. M. (2017). Viral disinfection of organic fresh produce comparing Polyphenon 60 from green tea with chlorine. *Food Control*, 79, 57-61. <http://dx.doi.org/10.1016/j.foodcont.2017.03.021>.
- Martins, C. S. F. (2018). *Filme ativo com extrato de chá verde na preservação de alimentos suscetíveis à oxidação lipídica* (Dissertação de mestrado). Universidade de Coimbra, Portugal.
- Michelin, D. C., Moreschi, P. E., Lima, A. C., Nascimento, G. G. F., Paganelli, M. O., & Chaud, M. V. (2005). Avaliação da atividade antimicrobiana de extratos vegetais. *Revista Brasileira de Farmacognosia*, 15(4), 316-320. <http://dx.doi.org/10.1590/S0102-695X2005000400010>.
- Montanari, R. M., Barbosa, L. C. A., Demuner, A. J., Silva, C. J., Andrade, N. J., Ismail, F. M. D., & Barbosa, M. C. A. (2012). Exposure to an acardiaceae volatile oils and their constituents induces lipid peroxidation within foodborne bacteria cells. *Molecules (Basel, Switzerland)*, 17(8), 9728-9740. <http://dx.doi.org/10.3390/molecules17089728>. PMID:22893019.
- Oliveira, A. B. A., Ritter, A. C., Tondo, E. C., & Cardoso, M. I. (2012). Comparison of different washing and disinfection protocols used by food services in Southern Brazil for lettuce (*Lactuca sativa*). *Food and Nutrition Sciences*, 3(1), 28-33. <http://dx.doi.org/10.4236/fns.2012.31006>.
- Ortiz-Solà, J., Abadias, M., Colás-Medà, P., Sánchez, G., Bobo, G., & Viñas, I. (2020). Evaluation of a sanitizing washing step with different chemical disinfectants for the strawberry processing industry. *International Journal of Food Microbiology*, 334, 108810. <http://dx.doi.org/10.1016/j.ijfoodmicro.2020.108810>. PMID:32805511.
- Özvrural, E. B., Huang, Q., & Chikindas, M. L. (2016). The comparison of quality and microbiological characteristics of hamburger patties enriched with green tea extract using three techniques: direct addition, edible coating, and encapsulation. *Food Science and Technology (Campinas)*, 68, 385-390.
- Patil, M. P., Patil, K. T., Ngabire, D., Seo, Y. B., & Kim, G. D. (2016). Phytochemical, antioxidant and antibacterial activity of black tea (*Camellia sinensis*). *International Journal of Pharmacognosy and Phytochemical Research*, 8(2), 341-346.
- Pelissari, E. M. R., Covre, K. V., Rosario, D. K. A., & São José, J. F. (2021). Application of chemometrics to assess the influence of ultrasound

- and chemical sanitizers on vegetables: Impact on natural microbiota, Salmonella Enteritidis and physicochemical nutritional quality. *Lebensmittel-Wissenschaft + Technologie*, 148, 111711. <http://dx.doi.org/10.1016/j.lwt.2021.111711>.
- Randazzo, W., Falcó, I., Aznar, R., & Sánchez, G. (2017). Effect of green tea extract on enteric viruses and its application as natural sanitizer. *Food Microbiology*, 66, 150-156. <http://dx.doi.org/10.1016/j.fm.2017.04.018>. PMID:28576363.
- Reygaert, W. C. (2014). The antimicrobial possibilities of green tea. *Frontiers in Microbiology*, 5, 434. <http://dx.doi.org/10.3389/fmicb.2014.00434>. PMID:25191312.
- Rocha, T. M., Gonçalves, E. D. B. A., & Faria, M. V. C. (2010). Lavagem e sanitização em maçã (*Malus domestica* BORKH) cultivar Royal Gala: avaliação na redução de pesticidas organofosforados. *Alimentos e Nutrição*, 21(4), 659-665.
- Rodgers, S. (2016). Minimally processed functional foods: technological and operational pathways. *Journal of Food Science*, 81(10), R2309-R2319. <http://dx.doi.org/10.1111/1750-3841.13422>. PMID:27603331.
- Rosário, D. K. A., Mutz, Y. S., Peixoto, J. M. C., Oliveira, S. B. S., Carvalho, R. V., Carneiro, J. C. S., São José, J. F. B., & Bernardes, P. C. (2017). Ultrasound improves chemical reduction of natural contaminant microbiota and *Salmonella enterica* subsp. *enterica* on strawberries. *International Journal of Food Microbiology*, 241, 23-29. <http://dx.doi.org/10.1016/j.ijfoodmicro.2016.10.009>. PMID:27743521.
- São José, J. F. B. (2017). Estratégias alternativas na higienização de frutas e hortaliças. *Revista de Ciências Agrárias (Belém)*, 40(3), 630-640.
- São José, J. F. B., & Vanetti, M. C. D. (2015). Application of ultrasound and chemical sanitizers to watercress, parsley and strawberry: microbiological and physicochemical quality. *Lebensmittel-Wissenschaft & Technologie*, 63, 946-952.
- São José, J. F. B., Medeiros, H. S., Bernardes, P. C., & Andrade, N. J. (2014). Removal of *Salmonella enterica* Enteritidis and *Escherichia coli* from green peppers and melons by ultrasound and organic acids. *International Journal of Food Microbiology*, 190, 9-13. <http://dx.doi.org/10.1016/j.ijfoodmicro.2014.08.015>. PMID:25173914.
- São José, J. F. B., Medeiros, H. S., Andrade, N. J., Ramos, A. M., & Vanetti, M. C. D. (2018). Effect of ultrasound and chemical compounds on microbial contamination, physicochemical parameters, and bioactive compounds of cherry tomatoes. *Italian Journal of Food Science*, 30(3), 467-486.
- Sharma, A., Gupta, S., Sarethy, I. P., Dang, S., & Gabrani, R. (2012). Green tea extract: possible mechanism and antibacterial activity on skin pathogens. *Food Chemistry*, 135(2), 672-675. <http://dx.doi.org/10.1016/j.foodchem.2012.04.143>. PMID:22868144.
- Verrillo, M., Salzano, M., Cozzolino, V., Spaccini, R., & Piccolo, A. (2021). Bioactivity and antimicrobial properties of chemically characterized compost teas from different green composts. *Waste Management (New York, N.Y.)*, 120, 98-107. <http://dx.doi.org/10.1016/j.wasman.2020.11.013>. PMID:33290882.
- Zhang, Z., Zhang, X., Bi, K., He, Y., Yan, W., Yang, C. S., & Zhang, J. (2021). Potential protective mechanisms of green tea polyphenol EGCG against COVID-19. *Trends in Food Science & Technology*, 114, 11-24. <http://dx.doi.org/10.1016/j.tifs.2021.05.023>. PMID:34054222.
- Zihadi, M. A. H., Rahman, M., Talukder, S., Hasan, M. M., Nahar, S., & Sikder, M. H. (2019). Antibacterial efficacy of ethanolic extract of *Camellia sinensis* and *Azadirachta indica* leaves on methicillin-resistant *Staphylococcus aureus* and shiga-toxigenic *Escherichia coli*. *Journal of Advanced Veterinary and Animal Research*, 6(2), 247-252. <http://dx.doi.org/10.5455/javar.2019.f340>. PMID:31453199.
- Wanasundara, U. N., & Shahidi, F. (1998). Antioxidant and pro-oxidant activity of green tea extracts in marine oils. *Food Chemistry*, 63(3), 335-342. [http://dx.doi.org/10.1016/S0308-8146\(98\)00025-9](http://dx.doi.org/10.1016/S0308-8146(98)00025-9).
- Wong-Leung, Y. L. (1988). Antibacterial activities of some Hong Kong plants used in Chinese medicine. *Fitoterapia*, 69(1), 11-16.