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Tahiti Lemon Juice: A Natural Alternative to Reduce Bacteria from Eggshells

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Abstract: Tahiti lemon juice (*Citrus aurantifolia*) was evaluated for its antibacterial activity. For this purpose, microbiological parameters were assessed in vitro (measurement of the inhibition potential of bacteria using the disk diffusion method) and in vivo (the number of total aerobic mesophilic bacteria and Enterobacteriaceae on the eggshell). Tahiti lemon juice inhibited *Escherichia coli* and *Staphylococcus aureus*, with larger halos for pure juice. The values were 14.33 ± 0.58 mm for *E. coli* and 16.00 ± 1.00 mm for *S. aureus*. Compared with the load of mesophilic bacteria in the shells of non-sanitized eggs (5.49 ± 0.12 $\log_{10}$ CFU/mL), Tahiti lemon juice significantly reduced this bacterial group on the eggshell (2.07 ± 0.18 $\log_{10}$ CFU/mL). Combining the characteristics inherent to Tahiti lemon juice and the sanitization processes of poultry products proved helpful in guaranteeing the bacterial quality of eggs.

Keywords: antibacterials; eggs; microbiology safety; natural products; poultry; sanitizers

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1. Introduction

Poultry raised in controlled and well-managed environments with temperature control, adequate ventilation, correct lighting, strict hygiene, balanced nutrition, continuous monitoring, and biosecurity measures still produces eggs with an initial bacterial load. Although the oviduct is not entirely sterile [1], it is typical for the bacterial load on eggshells to increase due to the interaction of the eggs with the external environment [2]. The bacterial diversity on egg surfaces can determine egg quality. Sanitizing the egg surface can ensure the microbiological quality of the egg's internal microenvironment [3].

Microbiological contamination of eggshells has triggered the testing of several antibacterial solutions over the last 10 years. A review by Oliveira et al. [4] showed that sanitizers such as formaldehyde, quaternary ammonium, peracetic acid, hydrogen peroxide, plant extracts, and essential oils, among others, have been evaluated. In this review, the authors highlight important insights into the effectiveness of sanitizing products and their implications for poultry and human health. This last point reflects on a growing trend of prioritizing natural raw materials to balance efficacy and poultry and human health. After the introduction of natural products in the poultry field, the antibacterial properties of fruit juices have been explored to sanitize eggshells [5,6].

The Tahiti lemon (*Citrus aurantifolia*) (Figure 1), belonging to the Rutaceae family, is a plant that produces a fruit appreciated in human consumption and in pharmaceutical and medical sectors [7]. This fruit, called Tahiti lemon, is composed predominantly of citric acid and is an organic tricarboxylic acid recognized for its bactericidal properties,

as reviewed by Coban [8]. Due to the benefits of citric acid, Tahiti lemon can be a viable and economically accessible raw material for egg sanitization. Therefore, the present study aimed to investigate the effectiveness of the in vitro antibacterial properties of Tahiti lemon juice and its effect on reducing the bacterial load on eggshell surfaces.

Figure 1. Photograph of *Citrus aurantifolia* plant.

2. Materials and Methods

Tahiti lemons were purchased from a commercial market and subjected to rigorous cleaning and sanitization. Initially, the fruits were washed in running water to remove any surface residue, followed by immersion in a sodium hypochlorite-based sanitizer, according to the standards established by the manufacturer (Mercotech, Uberaba, Minas Gerais, Brazil). Subsequently, the lemons were placed on a sanitized surface and allowed to dry for 30 min. Under aseptic conditions, the dried lemons were carefully peeled and cut into half. The fruits were then manually squeezed into a previously cleaned beaker to remove the juice. After completion of the extraction process, the juice was immediately strained into a second beaker using a fine sieve and used immediately.

Tahiti lemon juice was transferred to a beaker and subjected to triplicate pH analysis using a high-precision digital pH meter, model 206–pH2, manufactured by Testo® (Lenzkirch, Breisgau-Hochschwarzwald, Baden-Württemberg, Germany). The device was previously calibrated following the necessary protocols to ensure measurement accuracy [9].

The evaluation of the antibacterial activity of Tahiti lemon juice against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) (American Type Culture Collection (ATCC), Manassas, VA, USA) was conducted using the disk diffusion method [10]. This technique involved plating bacteria on solidified Mueller–Hinton agar plates. Sterile filter paper discs (6 mm in diameter) were placed on these plates and impregnated with ten µL of 17 different concentrations of Tahiti lemon juice (2400 to 0.037 mg/mL) or pure Tahiti lemon juice. Each plate contained three discs impregnated with Tahiti lemon juice at the same concentration, a positive control disc (azithromycin 15 µg; Laborclin, Pinhais, Paraná, Brazil), and a negative control disc (distilled water). The prepared plates were incubated at 36 °C for 24 h. After the incubation period, the diameters of the inhibition zones were measured using a manual caliper (Mitutoyo, Suzano, São Paulo, Brazil).

Unwashed eggs from Embrapa 051 hens were donated by a poultry production unit in compliance with animal welfare standards (Planaltina, Federal District, Brazil). Initially, clean shell eggs were selected. They were then subjected to candling to detect cracks and breaks, discarding those that showed any defects. Eggs were randomly identified, representing three distinct groups: non-sanitized eggs, eggs sanitized with 1% quaternary ammonium (Mercotech, Uberaba, Minas Gerais, Brazil), and eggs sanitized with Tahiti lemon juice at 1200 mg/mL (juice/water). Sanitizers were prepared and immediately

applied using a hand sprayer. After application, the eggs were allowed to dry for 30 min. Six eggs (one per bag) from each treatment were placed in sterile bags (Labplas, Sainte-Julie, Quebec, Canada) containing 75 mL of 0.1% peptone water (Laborclin, Pinhais, Paraná, Brazil). After washing the eggs with this solution for 2 min, the wash water was serially diluted. Subsequently, 100 µL of these dilutions was plated on plate count agar (Laborclin, Pinhais, Paraná, Brazil) to count mesophilic bacteria and on violet red bile glucose agar (Laborclin, Pinhais, Paraná, Brazil) to quantify Enterobacteriaceae. The plates were incubated at 36 °C for 48 h. After incubation, the number of colonies was counted, and the results were transformed into logarithmic values ($\log_{10}$).

We monitored the weight loss of 18 eggs subjected to different treatments (6 eggs/treatment) over 21 days to understand their potential influence on egg gas exchange and moisture. Eggs were weighed immediately after sanitization, and the same procedure was performed on non-sanitized eggs. All eggs were stored at room temperature. After 21 days of storage, the eggs were weighed again.

A completely randomized design was used with triplicate analyses for in vitro antibacterial analysis, six repetitions for eggshell bacterial counts, and six repetitions for evaluating egg weight loss. SAS Studio University Edition software (SAS Inst. Inc., Cary, NC, USA) was used to analyze variance (PROC GLM). The Tukey test was used ($p < 0.05$) to determine significant differences among means.

3. Results and Discussion

The bacterial properties of lemon-derived products, such as juice and essential oil, have been one of the gateways for researchers seeking natural and healthy alternatives for bacterial control on eggshell surfaces [11–14]. The results of these researchers pave the way for future research to consolidate the beneficial effects of lemon on the healthy management of eggs. In this study, we specifically focused on the effectiveness of Tahiti lemon juice as a sanitizer for chicken eggs.

The average pH of the Tahiti lemon was 2.42 ± 0.04, which is closely aligned with the pH value of Tahiti lemon described by Mohd-Hanif et al. [9], who reported a pH of 2.34 ± 0.01. In addition to citric acid, other bioactive compounds, such as flavonoids, cardiac glycosides, alkaloids, and terpenoids, can be found in Tahiti lemon juice, contributing to its biological versatility [15].

We investigated the antibacterial activity of 17 concentrations of Tahiti lemon juice in vitro. Our results revealed that only two of these concentrations, 2400 and 1200 mg/mL, along with pure Tahiti lemon juice, demonstrated significant inhibitory potential against both bacteria tested. Specifically, 2400 mg/mL produced inhibition zones of 11.67 ± 0.58 mm and 12.33 ± 1.53 mm for *E. coli* and *S. aureus*, respectively. Likewise, the 1200 mg/mL concentration resulted in zones of inhibition of 10.33 ± 1.15 mm for *E. coli* and 11.00 ± 1.73 mm for *S. aureus*. Pure Tahiti lemon juice had an inhibitory activity of 14.33 ± 0.58 mm against *E. coli* and 16.00 ± 1.00 mm against *S. aureus*. The antibacterial efficacy of Tahiti lemon juice (Figure 2), assessed by the same in vitro analysis method, has also been corroborated by several previous studies [15–18]. The consistency of the results obtained in this study with previous investigations was expected because citrus compounds exhibit a dual mechanism of action, encompassing physical and chemical effects [19]. Physically, citrus components cause significant disturbances in bacterial membranes, resulting in the leakage of vital intracellular components. Chemically, citrus interacts with the cell envelopes of bacteria, for example, by altering the carboxylic groups of fatty acids present in the membrane [19]. Considering that 1200 mg/mL was the lowest effective concentration against both bacteria, it was chosen to be tested in egg microbiology.

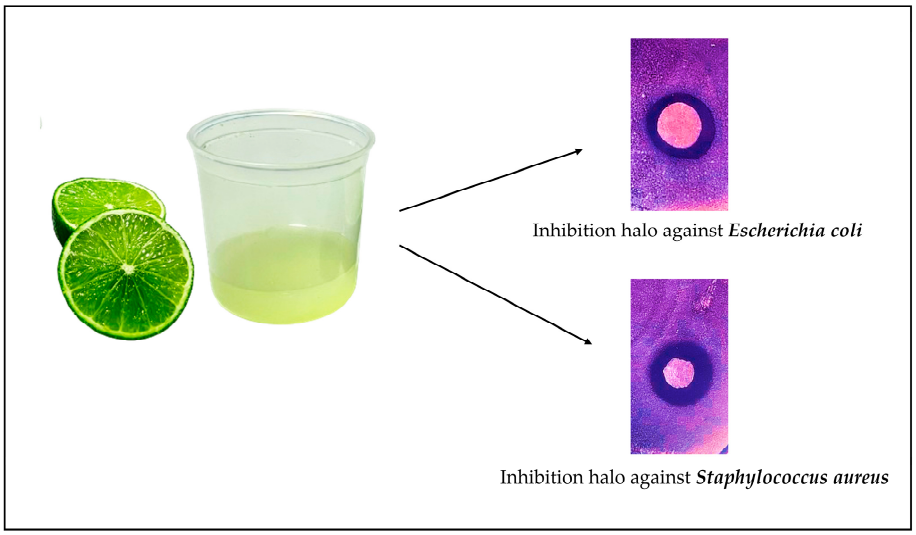

Figure 2. Antibacterial activity of Tahiti lemon juice against two bacterial strains.

Tahiti lemon juice notably reduced the bacterial load on eggshells compared with that on non-sanitized eggs ($p < 0.0001$), achieving a 3.42 $\log_{10}$ CFU/mL reduction (Table 1). The difference was also statistically significant compared with quaternary ammonium treatment, which resulted in an additional 1.05 $\log_{10}$ CFU/mL reduction ($p < 0.0001$). Microbiological analysis was started 30 min after sanitization, demonstrating Tahiti lemon juice's rapid action in the eggshells' bacterial protection. This short period highlights the immediate effectiveness of Tahiti lemon juice as an antibacterial agent. Corroborating the findings of the present study, Abdulwahid et al. [11] demonstrated that *Cronobacter sakazakii*, *Raoultella ornithinolytica*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Moraxella* spp., and *Serratia plymuthica* were no longer present on eggshells after disinfection with Tahiti lemon juice. In the Enterobacteriaceae analyses, mean values ranged from 0.00 ± 0.00 $\log_{10}$ CFU/mL for eggs sanitized with quaternary ammonium to 2.57 ± 0.51 $\log_{10}$ CFU/mL for non-sanitized eggs. The application of Tahiti lemon juice significantly reduced Enterobacteriaceae counts ($p < 0.0001$), which were comparable to those obtained with quaternary ammonium sanitization. Hygienic deficiencies in egg production facilities, as evidenced by bacteria from the Enterobacteriaceae group, can be effectively mitigated by sanitizing eggs with Tahiti lemon juice.

Table 1. Bacterial counts on surfaces of sanitized and non-sanitized eggshells [1].

Treatments	Total Aerobic Mesophilic Bacteria ($\log_{10}$ CFU/mL)	Enterobacteriaceae ($\log_{10}$ CFU/mL)
Non-sanitized eggs	5.49 ± 0.12 [a]	2.57 ± 0.51 [a]
Quaternary ammonium	1.02 ± 0.29 [c]	0.00 ± 0.00 [b]
Tahiti lemon juice	2.07 ± 0.18 [b]	0.65 ± 0.56 [b]
p value	<0.0001	<0.0001

[1] Data are expressed as mean ($\log_{10}$ CFU/mL) ± standard deviation. Different letters in same column indicate significant differences among means ($p < 0.05$).

It has been shown that sanitizers can compromise eggshell integrity, resulting in greater weight loss [20]. Likewise, forming a thick artificial film on the eggshell with certain sanitizers can significantly mitigate this loss [21]. These dynamics have different implications depending on the purpose of the eggs. Shell integrity is essential for hatching eggs, and any changes can harm the incubation process. In contrast, forming a protective film can be beneficial for table eggs and helps to preserve egg quality during storage. In this study, we observed that weight loss did not vary significantly between treatments ($p > 0.05$), maintaining an average of $6.42 \pm 0.09\%$. The data obtained suggest that the

treatments applied did not negatively affect eggshell integrity or quality. Even in the event of an effect on the shell, the magnitude of this impact was insufficient to compromise the essential water–gas exchange processes. This is essential for both hatching and table eggs and ensures that the quality of the final product is maintained regardless of the intended end use.

4. Conclusions

From an antibacterial perspective, Tahiti lemon juice effectively inhibited bacterial growth on plates using the disk diffusion method and eggshells, demonstrating an efficiency comparable to that of quaternary ammonium. This finding is relevant for the egg production industry for human consumption and chick production, and provides a sanitization option that is effective, natural, safe, accessible, versatile, and known worldwide. As the antibacterial properties of Tahiti lemon juice are evaluated and confirmed against other bacteria, we hope that its use will become an option for sanitizing eggs.

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References

1. Wen, C.; Li, Q.; Lan, F.; Li, X.; Li, G.; Yan, Y.; Wu, G.; Yang, N.; Sun, C. Microbiota Continuum Along the Chicken Oviduct And Its Association With Host Genetics And Egg Formation. *Poult. Sci.* **2021**, *100*, 101104. [CrossRef] [PubMed]
2. Cader, S.; Goburdhun, D.; Neetoo, H. Assessment of the Microbial Safety and Quality of Eggs from Small and Large-Scale Hen Breeders. *J. World's Poult. Res.* **2014**, *4*, 75–81.
3. Chan, H.Y.; Meor Hussin, A.S.; Ahmad, N.H.; Rukayadi, Y.; Farouk, A.E. Effectiveness of Quaternary Ammonium in Reducing Microbial Load on Eggs. *Molecules* **2021**, *26*, 5259. [CrossRef] [PubMed]
4. Oliveira, G.d.S.; McManus, C.; Salgado, C.B.; dos Santos, V.M. Effects of Sanitizers on Microbiological Control of Hatching Eggshells and Poultry Health during Embryogenesis and Early Stages after Hatching in the Last Decade. *Animals* **2022**, *12*, 2826. [CrossRef] [PubMed]
5. Batkowska, J.; Wlazlo, L.; Drabik, K.; Nowakowicz-Debek, B.; Al-Shammari, K.I.A.; Gryzinska, M. Evaluation of Grapefruit Juice (*Citrus paradisi*) as an Alternative Disinfectant for Hatching Eggs. *Pak. J. Zool.* **2018**, *50*, 647–653. [CrossRef]
6. Taşdemir, A.N.; Onbaşılar, E.E.; Yalçın, S.; Boyalı, B.; Aygören, H.; Tülek, E.; Sarıçam, S.; Akan, M. Effects of Oregano Juice on Eggshell Microbial Load, Layer Embryo Development, Hatching Results, and Growth at the First 2 Weeks after Hatch. *Trop. Anim. Health Prod.* **2021**, *53*, 404. [CrossRef] [PubMed]
7. Enejoh, O.S.; Ogunyemi, I.O.; Bala, M.S.; Oruene, I.S.; Suleiman, M.M.; Ambali, S.F. Ethnomedical Importance of *Citrus Aurantifolia* (Christm) Swingle. *Pharm. Innov. J.* **2015**, *4*, 1–6.
8. Coban, H.B. Organic Acids as Antimicrobial Food Agents: Applications and Microbial Productions. *Bioprocess Biosyst. Eng.* **2020**, *43*, 569–591. [CrossRef] [PubMed]
9. Mohd-Hanif, H.; Shamsudin, R.; Adzahan, N.M. UVC Dosage Effects on The Physico-Chemical Properties of Lime (*Citrus aurantifolia*) juice. *Food Sci. Biotechnol.* **2016**, *25*, 63–67. [CrossRef]
10. Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; Turck, M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am. J. Clin. Pathol.* **1966**, *45*, 493–496. [CrossRef]

11. Abdulwahid, A.T.; Nasear, H.A.; Ghazi, S.S. Disinfection of Table Eggs Using Lemon Juice as a Natural Biocide. *Basrah J. Vet. Res.* **2020**, *19*, 177–189.
12. Oliveira, G.D.S.; McManus, C.; Pires, P.G.D.S.; dos Santos, V.M. Combination of Cassava Starch Biopolymer and Essential Oils for Coating Table Eggs. *Front. Sustain. Food Syst.* **2022**, *6*, 957229. [CrossRef]
13. Oliveira, G.d.S.; McManus, C.; Salgado, C.B.; Pires, P.G.d.S.; de Figueiredo Sousa, H.A.; da Silva, E.R.; dos Santos, V.M. Antimicrobial Coating Based on Tahiti Lemon Essential Oil and Green Banana Flour to Preserve the Internal Quality of Quail Eggs. *Animals* **2023**, *13*, 2123. [CrossRef] [PubMed]
14. Oliveira, G.d.S.; McManus, C.; Vale, I.R.R.; Dos Santos, V.M. Obtaining Microbiologically Safe Hatching Eggs from Hatcheries: Using Essential Oils for Integrated Sanitization Strategies in Hatching Eggs, Poultry Houses and Poultry. *Pathogens* **2024**, *13*, 260. [CrossRef]
15. Jesumirhewe, C.; Omeke, N.M.; Ogunlowo, O.P. Comparison of Phytochemicals, Antioxidant and Antimicrobial Activity of Peels and Juices of Citrus Species. *Fudma J. Sci.* **2019**, *3*, 258–265.
16. Ugwu, C.C.; Mbah-Omeje, K.N.; Ezeugwu, R.I.; Onuorah, S.C.; Agbo, M.C. Antimicrobial Activities and Phytochemical Screening of *Citrus Aurantifoila* (Lime) Leaf Extracts and Fruit Juice on Some Microorganisms. *Int. J. Innov. Res. Dev.* **2018**, *7*, 135–142.
17. Shakya, A.; Luitel, B.; Kumari, P.; Devkota, R.; Dahal, P.R.; Chaudhary, R. Comparative study of Antibacterial Activity of Juice and Peel Extract of Citrus Fruits. *Tribhuvan Univ. J. Microbiol.* **2019**, *6*, 82–88. [CrossRef]
18. Izah, S.C.; Odubo, T.C. Effects of *Citrus aurantifolia* Fruit Juice on Selected Pathogens of Public Health Importance. *ES Food Agrofor.* **2023**, *11*, 829. [CrossRef]
19. Álvarez-Ordóñez, A.; Carvajal, A.; Arguello, H.; Martínez-Lobo, F.J.; Naharro, G.; Rubio, P. Antibacterial Activity and Mode of Action of a Commercial Citrus Fruit Extract. *J. Appl. Microbiol.* **2013**, *115*, 50–60. [CrossRef]
20. Li, X.; Anderson, D.; Rathgeber, B.; McLean, N.; MacIsaac, J. Fumigating Broiler Hatching Eggs with Lysozyme Product (Inovapure) to Reduce Eggshell Microbial Load. *Poult. Sci.* **2018**, *97*, 4252–4261. [CrossRef]
21. Oliveira, G.S.; dos Santos, V.M.; Nascimento, S.T.; Rodrigues, J.C. Alternative Sanitizers to Paraformaldehyde for Incubation of Fertile Eggs. *Poult. Sci.* **2020**, *99*, 2001–2006. [CrossRef] [PubMed]