Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Comparative analysis of plant-based marinades for controlling *Staphylococcus aureus* in refrigerated chicken breast

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ARTICLE INFO

Keywords

Antibacterial Effects

Bioactive Compounds

Food Preservation

Food Safety

Staphylococcus aureus

Received 29/01/2025

Accepted 22/03/2025

Available On-Line 01/04/2025

ABSTRACT

Plant-based marinades have emerged as a safe, cost-effective, and sustainable natural preservative substance for meat preservation. Therefore, this study investigated the antibacterial effect of plant-based marinades, papaya juice, tomato juice, and their combination, on *Staphylococcus aureus* in experimentally inoculated chicken breast samples stored at 4°C for 48 h. Forty breast cuts were divided into four groups (10 cuts per group) and inspected at six points. *S. aureus* was injected into each of the four groups at 2 mL/100 g from a 37 °C overnight culture. Inoculated samples were kept at 4 °C for 30 min to adhere cells to meat. Marination of breast cuts was conducted using tomato juice (T1), papaya juice (T2), and tomato-papaya juice (T3) marinated breast slices, while distilled water was utilized as a control. The control group, treated with distilled water, exhibited a significant ncrease ($P < 0.05$) in *S. aureus* counts from 6.84±0.11 to 8.74±0.17 $\log_{10}$ CFU/mL.Papaya juice marination resulted in a notable reduction in *S. aureus* counts, achieving a 12.96% decrease at 48 h. Tomato juice also demonstrated antibacterial effects, with a 21.02 % reduction in bacterial counts at 48 h. The combined treatment with papaya and tomato juices resulted in the reduction *in S. aureus* counts, achieving 10.09% decrease after 48 h. In conclusion, the tomato marinated group showed the lowest reduction in *S. aureus* count. The results underscore the potential of natural plant-based marinades as effective antibacterial agents for enhancing the safety of poultry products and catering to consumer demand for natural food additives. Further research should explore the mechanisms underlying the observed antibacterial effects and evaluate the efficacy of these treatments against a broader spectrum of foodborne pathogens to substantiate their applicability in food preservation strategies further.

1. INTRODUCTION

Staphylococcus aureus is a significant concern in refrigerated chicken breasts, with studies showing its presence and potential for growth under various conditions. Research indicates that *S. aureus* can contaminate chicken meat during processing, with plucking and evisceration being the primary stages of contamination (Notermans et al., 1982). The prevalence of methicillin-resistant *S. aureus* (MRSA) in broiler chicken meat has been reported, with some strains harboring enterotoxin genes capable of producing toxins at temperatures above 8°C (Abolghait et al., 2020).

Interestingly, *S. aureus* demonstrates adaptive responses during the production of chicken meat. Transcriptional profiling reveals increased expression of genes associated with protein synthesis, cofactor production, and amino acid transport, while showing reduced expression of stress and virulence genes (Dupre et al., 2019). This adaptation allows *S. aureus* to utilize nutrients from chicken meat efficiently. However, contradictory findings suggest that in some clinical MRSA strains, toxin gene expressions may be upregulated when grown on chicken breast, potentially increasing pathogenicity (Chung et al., 2020).

To mitigate *S. aureus* growth and ensure food safety, various strategies have been explored. The integration of plant-derived natural preservatives in chicken meat preservation would provide numerous advantages. It offers an environmentally sustainable alternative to synthetic additives, corresponding with the increasing demand for sustainable food production in the sector. Employing plant-derived preservatives can decrease production expenses and enhance supply chain efficiency, hence increasing profitability. Furthermore, it improves product quality, minimizes food waste, and guarantees safe and healthy products (Seidavi, et al., 2021). Recently, various plant-based products have been implemented as bio-preservatives in chicken meat. Tomato (*Solanum lycopersicum*) and papya fruit (*Carica papaya*) juices can be utilized as both antimicrobial agents and marinades for chicken meat preservation. To date, numerous studies have been carried out on the effects of fruit marinade on meat preservation and meat quality. Pomegranate juice-based marinades also demonstrated effectiveness in extending the shelf life of chicken breast fillets by retarding microbial growth (Lytou et al., 2017).

Therefore, the current study aimed to investigate the antibacterial effects of plant-based marinades, papaya juice, tomato juice, and their combination, on Staphylococcus *aureus* in experimentally inoculated chicken breast samples stored at 4°C for 48 h.

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2. MATERIAL AND METHODS

2.1. Experimental management and approval.
The methods employed in this work were authorized by the Institutional Animal Care and Use Committee Research Ethics number (BUFVTM) of Benha University's Faculty of Veterinary Medicine, with the number BUFVM 12-10-2024.

2.2. Chicken samples
Fresh, skinless, boneless chicken breast was obtained from a nearby poultry processing facility for one-hour post-slaughter and transported directly to the laboratory at Benha University's Department of Food Hygiene and Control in insulated polystyrene containers filled with ice flakes. Subsequently, chicken meat samples were refrigerated at 4 °C until required.
2.3. Preparation of Marinade Juice (MJ):
Fresh ripped tomatoes (*Solanum lycopersicum*) and papaya fruits (*Carica papaya L.*) were procured from a nearby grocery store. The fruits were washed and divided into four pieces. The flesh of each fruit was manually separated and blended in a mixer for 30 seconds. The freshly made juice was utilized for treatment purposes.

2.4. Preparation of pathogens and inoculums
Bacterial Strains: *S. aureus* ATCC strain (25923) was obtained from the Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt. A fresh working culture of *S. aureus* was produced, as described by Sabike et al. (2015). *S. aureus* was plated on Baird Parker agar (Merck, Darmstadt, Germany) for 48 hrs at 37 °C. After that, five colonies from the plate were cultured in trypticase soy broth (Oxoid) at 37 °C. Two milliliters of grown cells were centrifuged at 13,000 × g at 4 °C for 15 minutes using a 0.85% w/v sodium chloride solution. After the cells were suspended in 2 mL saline, a cell solution containing approximately 9 $\log_{10}$ CFU/mL, yielding an optical density measurement of 0.1 at 625 nm as determined by a spectrophotometer.
2.5. Breast meat inoculation and marination
The experiment was carried out with a total of 40 breast cuts, each averaging 50 g, categorized into four groups (10 cuts per group) across six inspection points. *S. aureus* was injected into each of the four groups at a rate of 2 mL/100 g from an overnight culture at 37 °C and serially adjusted to 5 $\log_{10}$ CFU/mL (Sabike et al., 2015). The inoculated samples were stored at 4 °C for 30 min. to allow for cell attachment to the meat surface. Marination of breast cuts was conducted using tomato juice (T1), papaya juice (T2), and combined tomato and papaya juice (T3), and samples treated with distilled water were used as controls (Con). The concentration of marinade was selected based on findings from experimental pilot trials, where it exhibited optimal efficacy under the applied conditions. This concentration was deemed appropriate for the current investigation to ensure consistency and reliability of the obtained results. The marination was performed at a concentration of 1:1 (v/w) (marinade juice/meat cut) overnight at 4 °C. All samples were left on the sieve to dry before packing them in transparent polyethylene bags and finally storing them in cooled incubators at 4 °C for different intervals of time (0, 6, 12, 18, 24, and 48 h). These samples were used for the determinations of pH and *S. aureus* count.

2.6. pH analysis:
Approximately 2.5 g of breast meat was homogenized for 30 sec. using a Stomacher 400R (Seward, UK) in 25 mL sterile distilled water. The pH was measured using a calibrated pH meter (Jenway 3510; Cole-Parmer, Stone, UK).

2.7. Bacteriological Analysis
Bacteriological analysis was conducted during storage to determine the cell density of inoculated *S. aureus*. Each group's breast, weighing approximately 50 g, was deposited in sterile stomacher bags and mixed with a sterile physiological solution in a 1:2 (w/w) ratio. The mixture was then homogenized in a Stomacher 400R (Seward, UK) for 2 min, followed by serial dilution with physiological solutions. An appropriate inoculum from two different serially diluted *S. aureus* inoculated chicken breast cuts was plated over the surface of sterile Petri dishes containing Baird-Parker Agar base (BPA, Oxoid Ltd., UK) and Egg Yolk Tellurite Emulsion (SR0054, Oxoid Ltd., UK). The plates were subsequently incubated at 37 °C for 48 h (ISO, 2003).

2.8. Statistical analysis
SPSS Version 22 (SPSS Inc., Chicago, IL, USA) was used to analyze the data using one-way ANOVA. The statistical model compared the means using Tukey's multiple comparison test. A *P-value* of less than 0.05 was considered to indicate a significant difference.

3. RESULTS

The results illustrated the effect of plant-based marinades (papaya juice, tomato juice, and their combination) on the growth and reduction of *S. aureus* in experimentally inoculated chicken breast samples stored at 4°C for 48 h (Fig 1). Control samples, treated only with distilled water, exhibited consistent bacterial growth, whereas marinated samples demonstrated varying degrees of bacterial reduction, underscoring the antibacterial potential of the selected plant extracts. The control group, devoid of any plant extract treatment, showed a progressive increase in *S. aureus* counts throughout the 48-hour. The initial count of 6.84±0.11 $\log_{10}$ CFU/mL increased to 8.74±0.17 $\log_{10}$ CFU/mL by the end of storage (Fig 1).

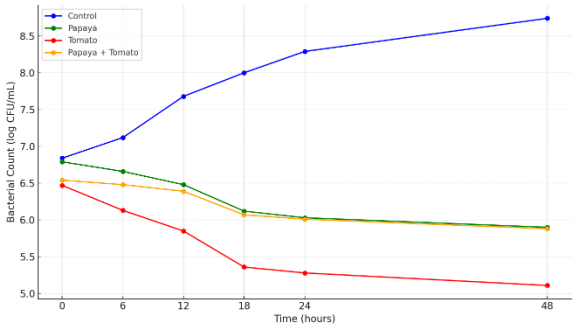

Fig 1 Effect of plant extract marination (Papaya, Tomato, and their combination) on *Staphylococcus aureus* counts in chicken breast over 48 h.

Papaya juice exhibited considerable antibacterial activity, with evident reductions in bacterial counts across all inspection points. The highest reduction (12.96%) was observed at 48 h, when the bacterial count decreased to 5.9±0.3 $\log_{10}$ CFU/mL compared to the control (Fig. 2). Tomato juice also demonstrated a significant antibacterial effect, achieved the greatest bacterial reduction, particularly noticeable at 48 h, with 21.02% decrease in bacterial count (5.11±1.05 $\log_{10}$ CFU/mL) (Fig 2). The combined treatment (papaya + tomato juice) showed a significant reduction. The count reduced from an initial 6.47±1.05 log10 CFU/mL to 5.88±0.23 log10 CFU/mL at 48 hours, resulting in a 10.09% reduction (Fig 2).

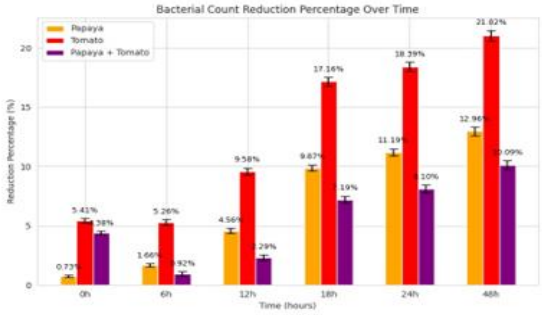

Fig 2 Percentage of reduction *S. aureus* in chicken breast

Table 1 pH measurement of chicken breast meat after marination for 48h.

Storage Time (hrs)	Control	Papaya	Tomato	Papaya + tomato
0	5.16±01.17	5.19±1.01	5.61±1.19	5.27±1.21
6	5.21±0.02	5.29±0.06	5.48±1.22	5.27±0.01
12	5.61±0.1	5.5±0.02	4.67±0.1	4.86±1.39
18	5.43±1.01	4.9±0.02	4.67±0.21	4.5±0.01
24	4.93±1.11	4.53±0.51	4.26±0.1	4.4±0.25
48	4.73±0.2	4.03±0.16	4.01±0.5	4.19±0.1

4. DISCUSSION

This study investigated the antibacterial effects of papaya juice, tomato juice, and their combination on *S. aureus* in inoculated chicken breast samples stored at 4 °C for 48 h. The control group, treated with distilled water, exhibited a significant increase in bacterial counts from 6.84±0.11 to 8.74±0.17 $\log_{10}$ CFU/mL, indicating favorable conditions for bacterial proliferation in untreated meat.

Papaya juice marination resulted in a notable reduction in *S. aureus* counts, with 12.96% decrease after 48 h. This antibacterial activity can be attributed to the bioactive compounds present in papaya, such as alkaloids and flavonoids, which have been documented for their antibacterial properties (Koul et al., 2022). These findings align with the existing literature on the antimicrobial properties of papaya and its efficacy against pathogens in chicken meat (Sugiharto, 2020). Radiastuti et al. (2020) evaluated the use of papaya seed powder as a preservative in chicken meat, observing a decrease in bacterial growth, including *S. aureus*, thereby extending the meat's shelf life.

Tomato juice also demonstrated antibacterial effects, with 21.02% reduction in bacterial count after 48 h. The antimicrobial properties of tomatoes are linked to their biochemical composition, including phenolic compounds and organic acids, which have been shown to inhibit the growth of Grame-positive bacteria, such as *S. aureus* (Szabo et al., 2019). Fermented tomato byproduct extracts exhibit antimicrobial activity against various pathogens, including *Staphylococcus aureus*. A previous study found that tomato byproduct extracts had the highest activity against *E. coli*, *S. aureus*, *Bacillus cereus*, and Pseudomonas spp. (Ricci et al., 2021). Furthermore, tomato juice sonication contributed to a reduction in the number of analyzed microorganisms, indicating its potential antimicrobial effects (Starek et al., 2021). Isidro-Requejo et al. (2023) discussed the benefits of tomato plant extracts, including their antimicrobial activity, which can be harnessed to improve food preservation. Ruíz-Cruz et al. (2018) examined the application of edible chitosan-tomato plant extract coatings on chicken fillets. The study found that this treatment effectively inhibited the growth of *S. aureus*, enhancing microbiological safety and extending the shelf life of meat during refrigerated storage.

The combined treatment with papaya and tomato juices resulted in the most significant reduction in *S. aureus* counts, achieving a 10.09% decrease by 48 h. The plant-derived antimicrobials have been explored in various studies, highlighting the potential of combining different plant extracts to enhance antimicrobial effects (Karnwal and Malik, 2024). These results underscore the potential of natural plant-based marinades as effective antibacterial agents for enhancing the safety of poultry products. While tomato juice alone showed the highest efficacy among the individual treatments, its combination with tomato juice amplified the reduction, likely due to the interaction of their bioactive constituents.

Therefore, the use of natural antimicrobials such as papaya and tomato juices offer a promising alternative to synthetic preservatives, catering to the consumer demand for natural food additives and contributing to improved food safety.

5. CONCLUSIONS

In conclusion, all plant marinades significantly affected the *S. aureus* count in experimentally inoculated chicken, with tomato juice demonstrating the most pronounced drop in *S. aureus* count after 48 hours. The findings suggest that plant-derived natural marinades may function as effective antibacterial agents, thereby enhancing the safety of poultry products. Further investigation is required to clarify the precise mechanisms behind the observed antimicrobial effects and to evaluate the impact of these treatments on the sensory attributes and nutritional quality of food products.

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