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Microbiological characteristics of minced Beef treated with Lemon Juice (Citric acid)

Mariam A. Jalil & Raad J. Hammadi

Department of Public Health, College of Veterinary Medicine, University of Diyala, Iraq.
Corresponding E-Mail: raad.j@uodiyala.edu.iq

ORCID : https//orcid.org/0009-0005-8916-2536

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Abstract:

Meat can undergo many changes and Considers a perfect media for a large number of pathogenic and putrefaction microorganisms during primary and further processing. Several techniques are depended for food preservation, including chemical, physical as well as biological methods., The use of food preservatives to prevent microbial growth is one of the most common and effective approaches to ensuring food safety. However, growing of pathogenic bacteria over the health risks associated with chemical preservatives have increased interest in natural alternatives. Natural preservatives exhibit potent antimicrobial activity while significantly reducing potential health risks.

The goal of this study is to check the impacts of lemon of lemon juice (citric acid) on the total bacterial count in minced meat of beef and the computational efficiency of bacterial systems to improve that sanitary quality of minced beef meat before cooking and eating. Inactivation of total bacterial count by lemon juice (citric acid) may provide the practical with simple method to ensuring beef minced safety food.

Minced beef samples were separately inoculated with high levels of each bacterial count, then treated with various concentration of fresh citric acid (2.5%, 5%, 7.5% with 10 %) over multiple exposure times (3, 6, 12 and 24 hrs).

The results revealed that adding citric acid in different concentrations led to a reduction in total bacteria count of between (41.3%) and (19.8%) log CFU/ml, while adding lemon juice in different concentrations and times resulted in a reduction of between 19.6 and 12 log CFU/ml. Citric acid demonstrated concentration- and time- dependent antibacterial activity, showing minimal early inhibition at low levels but increasing efficacy over extended duration and higher concentration.

Keywords: Minced beef, lemon juice, citric acid

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Introduction

Meat products serve as vital sources of protein, B vitamins, minerals, essential amino acids, fat, , and various further nutrients (Hadidi, M. *et al.*, 2022). However, they may also contain potential hazards such as bacteria, fungi, chemical residues, and foreign contaminants (Faiz, M. *et al*., 2024). Meat that nutrient-rich globally considered the preferred as the animal protein source for a lot of people (Heinz and Hautzinger, 2007). Citrus and there waste are good sources as well as valued technological and nutritious properties can be utilized as functional constituents when scheming healthy recommended foods (functional recommended foods) (Marı´n, et al., 2002; Puupponen-Pimia et al., 2002).They have in height contented of bioactive combinations and can be utilized as natural additives of food, thickener agents, flavorings, colorants, antimicrobials, and antioxidants (Ayala-Zavala *et al*., 2011). the presence of synthetic additives in meat and its products raises concerns regarding potential health risks. As a result, meat producers and researchers are increasingly exploring natural alternatives that offer effective antimicrobial properties while minimizing adverse health effects (Yong, H.I. *et al*., 2021).

The antibacterial properties of citric acid were examined. To achieve the appropriate shelf life, many food items must be protected from deterioration throughout manufacturing, store, and delivery. Food quality and safety are endangered by slightly treated, quickly prepared, and easy-to-eat foodstuffs, also the worldwide trade for food as well as supply from centralized production centers (Lucera *et al.*, 2012). Besides, the growth of spoilage and pathogenic microorganisms can be managed through various food preservation techniques, which help extend shelf life and maintain food safety. These methods include chemical preservation using additives, physical approaches such as heat treatment, drying, freezing, and packaging, as well as biological preservation, which involves the use of beneficial microorganisms that inhibit harmful bacteria and produce bacteriocins (Arokiyaraj, S. *et al*., 2024).

Microbiological and/or biological substances may reduce the quality of food products throughout their preparation, storage, and distribution, which appear to be a major factor impacting their value, availability, and pricing. Foodborne illness can result in serious public health problems as well as financial losses due to food illness and meat rotting (Kingchaiyaphun & Rachtanapun, 2012). As a result, the use of appropriate antioxidant and antibacterial compounds may be beneficial for preserving meat quality, prolonging shelf life, and avoiding financial loss (Yin & Cheng, 2003).

There have been several efforts to identify natural remedies to prevent microbial contamination, also toward limit the process of oxidation in foodstuffs. Numerous studies found the antioxidant enzymes with microbial development inside meats (plants or herbs) that

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include antioxidants are well tolerated by humans (Flores & Toldra, 2011). Phyto extracts were screened, and the most of them included phenolic chemicals as secondary metabolites. Because of the high reducing nature groups of free hydroxyl (antioxidant characteristic) and ability binding of protein in these molecules, they have enormous biological relevance (Vaithiyanathan *et al.*, 2011).

The researcher (Chaisawadi *et al.*, 2003) reported antimicrobial activity in various therapeutic herbs and culinary items (e.g. sweet basil, lemongrass, ginger, coriander and, among others). There are conflicting data on whether citric acid wipes kill germs more effectively than lactic acid washes. For anstance, lactic acid (2%) decreased *E. coli* O157:H7 on muscle carcass of beef by (3.3) logs, while acid of citric (lemon) (5%) decreased it by (1.6) logs (Ransom *et al.*, 2003). It is indeed worth noting that the availability of healthcare herbs (i.e. plants that contain phytonutrients) has started to rise (Vaithiyanathan *et al.*, 2011).

Such researchers also discovered that spray or immersion treatments of citric acid with lactic acid on flesh of cheek showed that (1.1) log decreases in bacterial total. Organic acids' method about action in microbial cells isn't totally understood, although it's thought that the unreacted molecule of the acid is responsible for antibacterial activity. In regards of the cited decrease that can be accomplished, there is a lot of variation in the research. This is primarily due to variances in acidity utilized by different studies, application methods, and sample types examined (Cherrinton *et al.*, 1991; Ammor *et al.*, 2006).

The breakdown in constructions caused by enlargement of meat and greater converting of the collagen to the gelatin at lowering of pH during roasting is thought to be the process of tenderizing impact of acidic marinades (Offer & Knight, 1988). The study aimed to examine the antimicrobial and antibacterial activity of lemon juice, as well as to determine the best agents added to beef minced meat to prevent the microbiological and oxidative degradation.

Materials and methods

Sample preparation:

Beef minced meat was bought from a local butcher shops after the animal was slaughtering, dressing, and deboning. The samples were minced and placed in polyethylene bags, which were kept in the fridge overnight at 4 °C. Lemon juice was purchased from a nearby market. The lemon was split in a half then squeezed using a handly-pressed juice applicator (Kisla, 2008), then the pH was determined using a pH meter. Lemon fruit (citric acid) was kept refrigerated at 4°C in sealed bags. Lemons, which were employed in this study, are high in antioxidant and antibacterial compounds. Fresh lemon juice was acquired from a local market, and the cirtic acid was made.

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Minced meat preparation:

A 100 g sample of minced beef was divided into four equal portions (25 g). Four different concentrations of lemon juice was added to contaminated minced beef flesh (.the initial bacterial total count).. The other procedures were made by mixing the optimum quantities of the tested additions (2.5%, 5%, 7.5%, 10%), lemon juice with minced beef flesh and immersing it for varying lengths of time (3, 6,12, 24) hrs. Minced beef meat was placed in plastic plates, coated by polyethylene film, then stored at 4 ^{0}C inside the fridge. In minced beef meat, the influence of optimal concentration of this test application for lemon juice pH, with stander plate count (SPC) were evaluated during immersion time at 4 ^{0}C.

Standard plate count:

The standard plate count (SPC) method was used to evaluate total viable bacterial populations in the food calculated on nutrient agar, as suggested by the American Public Health Association (APHA 1992). Serial of dilutions were (10^{-3},10^{-4},10^{-5},10^{-6},10^{-7},10^{-8},10^{-9},10^{-10},10^{-11}) of the samples then placed on plates with incubated at 37 ^{0}C for 24-48 hours, as described by (Thunberg *et al.*, 2002).

Inoculation of minced meat:

A 25g of minced beef was added to 225 ml of distant water then homogenized manually. Subsequently, 0.1 m of serial dilution was put to plates count agar then incubated at 37°C for 24 hours (10^{-3},10^{-4},10^{-5},10^{-6},10^{-7},10^{-8},10^{-9},10^{-10},10^{-11}). The first microbial growth appeared, and the colonies were counted (Aymerich *et al.*, 2008).

Determining of pH values:

A beef meat minced (25) g was soaked with lemon juice for three hours, six hours, twelve hours, and twenty-four hours, then pH was determined with pH meter. Each of the four ground beef counts had three trials collected (Zhang *et al.*, 2021).

Results:

The initial pH value of the minced meat products was (5.8), and that of the lemon juice was (2.54), following the application of lemon juice, the average PH of the mixture reached (5.3) and subsequently decreased to (4.6) by the end of the trials. In contrast, treatment with citric acid resulted in an average PH of (4.3), which decreased to (3.8) by the end of study. Figure (1) illustrates the pH values of meat treated with different leves of citric acid across various time point.

Figure 2 to Figure 5 illustrast the clear effects of various concentrations of lemon juice (2.5%, 5%, 7.5%, and 10%) on total bacterial count inoculated into minced beef at

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various exposure times (3, 6, 12, and 24 hours). The low pH value of treated meat inhibits bacterial activity, thereby preventing the three main mechanisms of meat decay: which are microbial spoilage, lipid oxidation, and autoenzymatic spoilage.

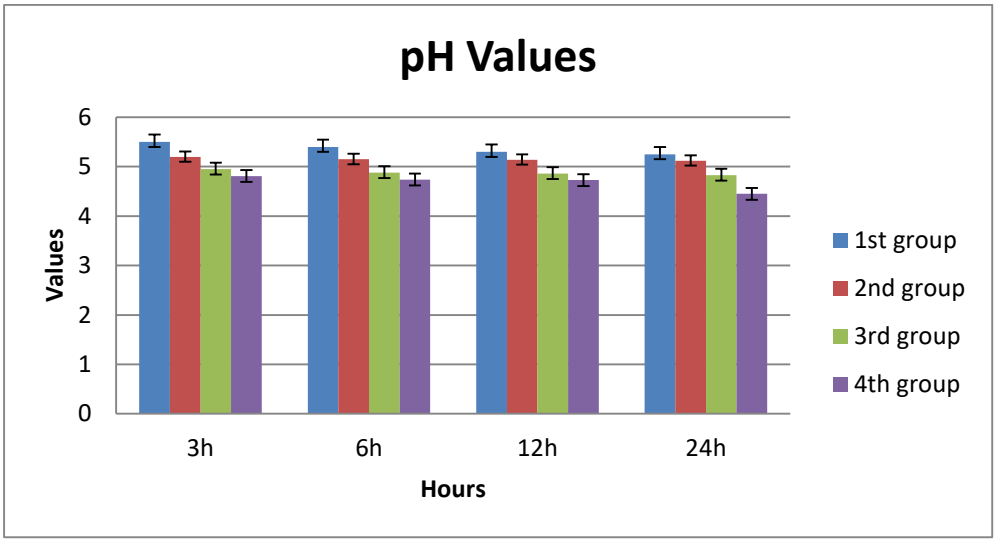

Figure 1: PH values of meat treated with different leves of citric acid across various time point.

When minced beef was soaked in various concentrations of the lemon juice, the decline in the initial bacterial total count across the treatment groups ranged from (41.3) to (36.4), (35.4) to (25.6), (25.5) to (22.1), and (21.3) to (19.8) log CFU/ml, (respectively) at different exposure times. Figurs (2 to 5) show the impacts of multiple citric acid concentrations (2.5%, 5%, 7.5%, and 10%) on the total bacteria count inoculated into minced beef at various exposure times (3, 6, 12, and 24 hours). Across these exposure times, the reduction in the initial total bacterial count in the groups ranged from (19.6) to (19.0), (18.2) to (17.5), (17.4) to (15.8), and (14.1) to (12.0) log CFU/ml, respectively.

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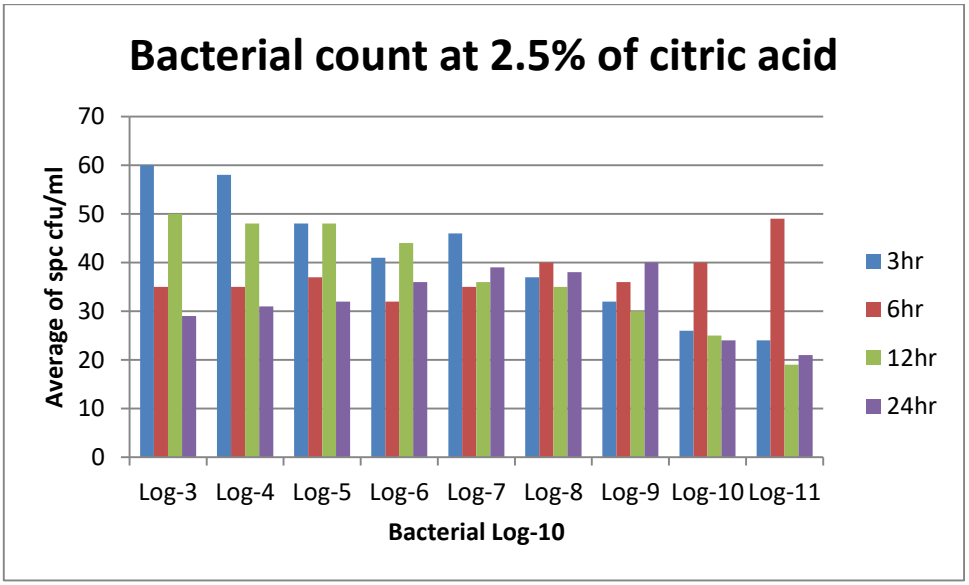

Figure (2): Bacterial count at 2.5% concentration & different times in lemon juice (citric acid).

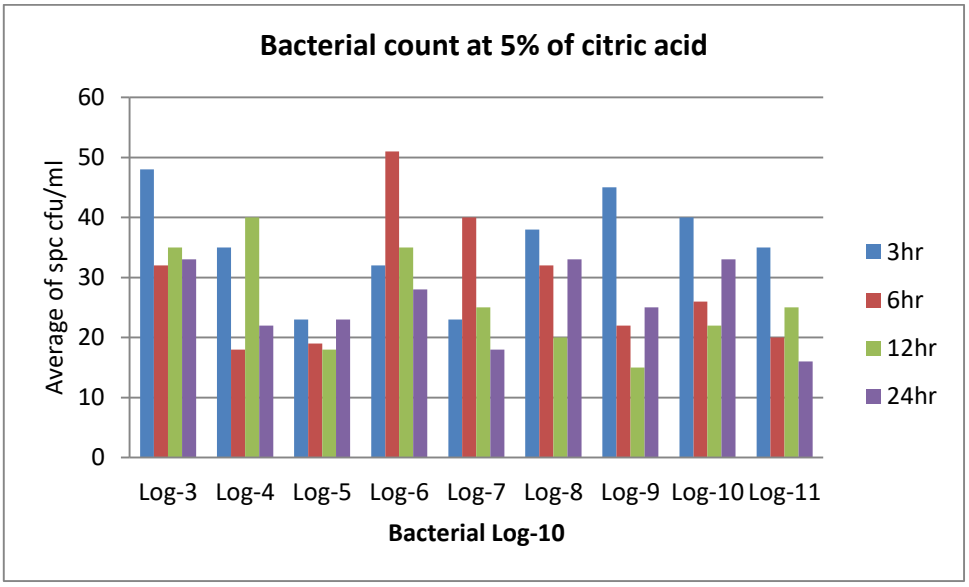

Figure (3): Bacterial count at 5% concentration & different times in lemon juice (citric acid).

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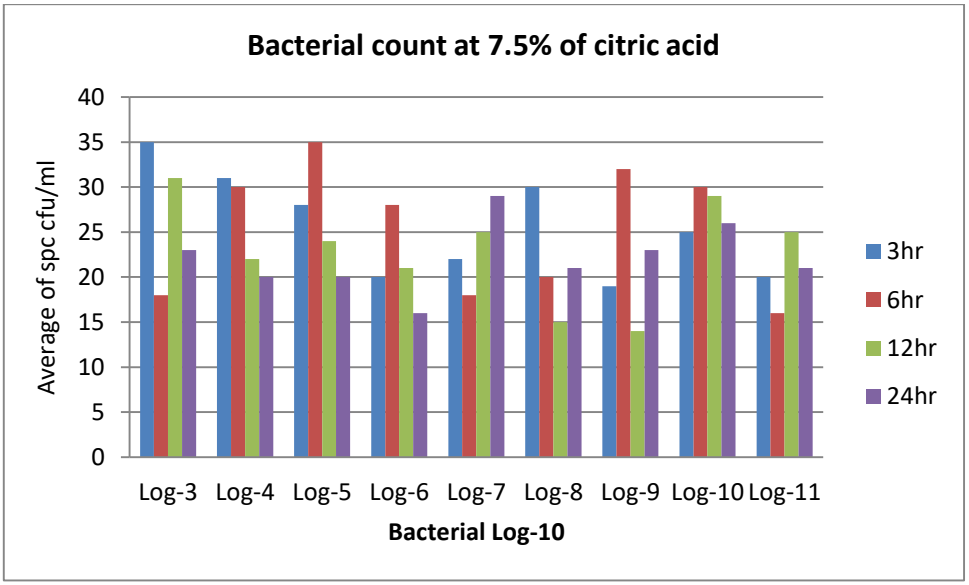

Figure (4): Bacterial count at 7.5% concentration & different times in lemon juice (citric acid).

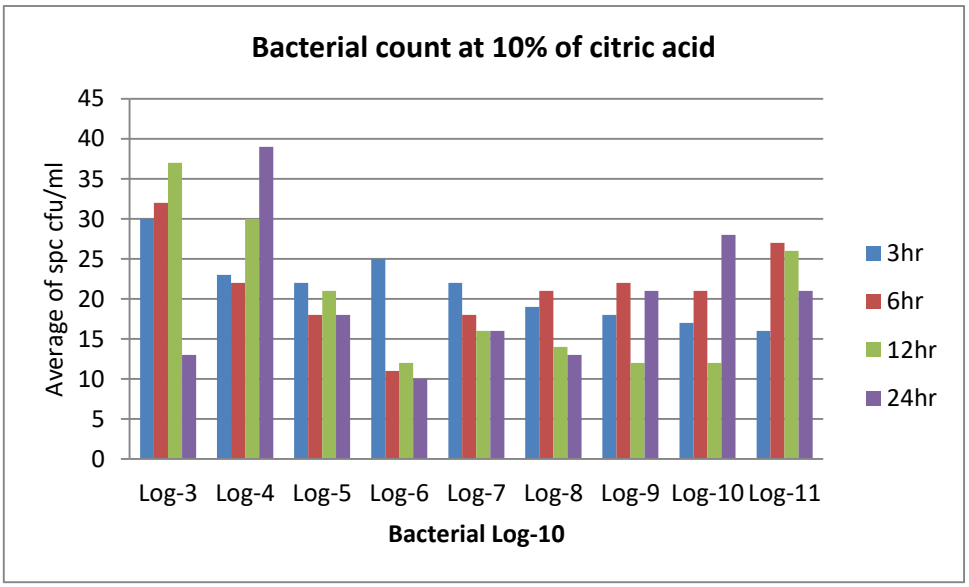

Figure (5): Bacterial count at 10% concentration & different times in lemon juice (citric acid).

Discussion:

Several studies have evaluated the antimicrobial and antioxidant effects of lemon solution in different meat products. According to the results of this study, Figures (1,to 5), increasing the citric acid concentration and the extending exposure time had a significant antimicrobial effect, leading to areducting in the total bacterial count in the minced beef.

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The presence of acid solution in various concentrations in household items such as lemon dressing and lemon juice supported the theory that the products might be utilized as antibacterial agents at homemade for minced meat (Kisla, 2008).

Natural compounds and their mixtures my be considered potential antimicrobial agents for preventing outbreaks of foodborne illness associated with fresh products at home, (Berge *et al.*, 2001). Furthermore, soaking minced beef in lemon juice for (24) hours can prevent various types of bacterial contamination and effectively decrease the microbial count on the meat (Yang *et al.*, 2013). In addition, Lemon essential oil has significant interest as a natural preservative for meat products due to its strong antimicrobial and antioxidant properties. Its incorporation aims to improve food safety and extend shelf life by inhibiting microbial growth and slowing oxidative processes. Lemon essential oil can be applied to meat products through various methods, including direct addition, incorporation into edible coatings, or as part of actve packaging films. Recent studies have explored the development of lemon nanoemulsions for meat preservation, using high-power ultrasonication for their formulation. Their application has shown promising potential to enhance the shelf life and quality of meat products (Zou, B. et al., 2023).

Regarding the inhibitory Effect Consumers prefer to consume raw meat after adding various concentrations of lemon juice. The resulting acidic environment provides a convenient and practical solution for enhancing the food safety of minced meat. Such treatments serve as effective methods to reduce the number of prevailing microorganisms. The study demonstrated that lemon juice significantly reduces microorganisms as arapid inhibitor, with its efficacy increasing over time and at higher concentration. The present finding indicate that the inhibitory activity of lemon juice strengthens with increased time and concentration, which is consistent with the results repoted Sengun et al. (2021). These results are in agreement with Xi et al. (2012), who reported that mixtures containing varying levels of lime powder, alone or combined with cranberry powder (pH 2.2) reduced the pH of naturally cured franks. These finding align with Bae et al, (2021), who reported that treatments containing lemon extract powder significantly reduced an aerobic plate counts (APC; $p<0.05$) relative to the control, furthermore, they indicated that vinegar powder could serve as a promising alternative to enhance nitrite functions in naturally cured sausages. These results also align with finding by Ben Hsouna, A. et al. (2017) who demonstrated that incorporating lemon extract as an additive at concentrations of 0.06 and 0.312 mg/g effectively prevent contamination and inhibits pathogenic bacterial growth, This antimicrobial action is attributed to the lipophilic nature of lemon compound, which disrupt bacterial cell membranes.

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Conclusions:

This study evaluated the effectiveness of adding lemon (citric acid) at various concentrations to enhance the quality of minced beef both before and after cooking. Consequently, lemon extract can serve as an effective natural preservative to inhibit various pathogenic bacteria in place of synthetic additives.Lemon act as powerful antimicrobial and antioxidant agents in meat preservation, primarily due to their rich phytochemical content.

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Conflict of Interest:

The author declares no conflict of interest regarding the publication of this research.

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This research did not receive external funding.

Authors Contributions:

The author was solely responsible for the conception, design, sample collection, laboratory experiments, data analysis, and manuscript preparation of this study.

Recommendations:

The application of lemon (citric acid) as a natural food faviorate and additive is recommended due to its of its efficacy in reducing bacterial contamination, improving meat quality, and ensuring safty for human consumption.Further research should evaluate other food additives to assess thir potential in minimizing contamination and enhancing product quality.However, excessive cocentrations of food additives and flavorings should be avoided to prevent adverse effects on consumer health.Future studies ought to explore synergistic combination with other natural preservatives and leverage advanced preservation technologies to enhance food safty and extend shelf lif without compromising sensory attributes.

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