



## Enhancing Microbiological and Histological Quality of Frozen Turkey Meat Using Vinegar

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### Abstract

**F**OODBORNE pathogens are health-threatening for humans. Therefore, appropriate methods to prevent foodborne illness and improve the health of human consumers have become necessary. In this study, the ability of vinegar to improve meat quality was assessed. The turkey meats were dipped for 20 minutes in 0, 1, 3, and 5% acidity dilutions of vinegar. The aerobic plate count (APC), coliform count (CC), and *Staphylococcus* count (SC) of the meat samples were then tested. Sensory evaluation and histological observation of muscle fibers were performed. The mean APC counts, CC values, and SC counts were significantly reduced at 5% acidity dilution of vinegar ( $P \leq 0.05$ ). Histologically, the skeletal muscle striations were preserved, especially after dipping meat in 5% vinegar. Additionally, sensory evaluation confirmed the ability of vinegar to improve turkey meat texture and taste. Based on our results, it is advised to increase the safety of turkey meat by dipping it in 5% vinegar for 20 minutes. Thus, vinegar is a straightforward, economical, safe, and highly effective method for meat decontamination.

**Keywords:** Aerobic plate count, Coliforms, Decontamination, Poultry meat, *Staphylococcus aureus*.

### Introduction

Because meat is a valuable source of protein, fat, vitamins, and minerals, it is inhabited by various microbes [1]. Depending on the pH, texture, storage conditions, temperature, and mode of transportation of the raw meat, different types of these microbes may survive and infect consumers [1, 2]. Foodborne diseases caused by the consumption of contaminated food have relevant public health implications [3]. In addition, it results in the annual condemnation of large quantities of food [4]. *Staphylococcus aureus*, and coliforms are common contaminants of several foods [5, 6]. Numerous methods have been employed to manage meat deterioration and microbiological contamination [7, 8]. Preservatives are defined as substances that can prolong the shelf life of different foods by protecting them against spoilage caused by microorganisms and/or protecting them against the

growth of pathogenic microorganisms [2]. The use of natural antimicrobial agents (natural preservatives) is environmentally safe, cost effective, and considered a tool for controlling microbial contamination [9]. Therefore, weak organic acids such as acetic acid and vinegar are frequently used as antimicrobial preservatives [10-12].

Recently, the use of natural antioxidants and antimicrobial agents for the preservation of chicken meat has attracted the attention of consumers because of their safety and potential health benefits [13, 14]. Vinegar has been used for generations for its ability to tenderize, preserve, enhance flavor, and even affect color [15]. Because vinegar contains acetic acid, it can lower the pH, which has strong antibacterial effects. It can dissociate inside microbial cells and alter metabolic mechanisms in microbes by lowering the pH [16].

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Numerous varieties of commercial vinegar are frequently utilized in marinades for meat and poultry [17, 18]. Freezing with vinegar is an excellent technique for preserving meat in which the meat can be preserved in a condition similar to that of a normal state and can be kept satisfactory for six months to one year [17, 19]. Thus, the goal of the current study was to assess the best dilution of vinegar for antimicrobial activity and the most valuable time for turkey meat decontamination.

## **Material and Methods**

### *Samples*

In the present investigation, 4-kilo panne of frozen turkey meat was purchased from same hypermarket. Turkey meat was transferred to the laboratory in an icebox under complete aseptic conditions without undue delay. The purchased turkey meat was divided into four groups, each with 1 kilogram of turkey meat. The acidity percentage of 5% of vinegar was used to assess its ability to improve meat quality. First group (G1) was considered the control group (the vinegar was not added). The second (G2), third (G3), and fourth (G4) groups were dipped for 20 minutes into 1, 3, and 5% acidity dilutions of vinegar, respectively, at room temperature. The experiments performed in this study were repeated three times to confirm our results.

### *Preparation of vinegar dilutions:*

In these experiments, vinegar from El-Naser Phar. Co. (96% acetic acid) with an initial acetic acid concentration of 5% was used. The vinegar was diluted with sterile distilled water to obtain the required acidity dilutions.

- 1- Vinegar (1%): added 20 ml of vinegar + 80 ml of sterile distilled water.
- 2- Vinegar (3%): added 60 ml of vinegar + 40 ml of sterile distilled water.
- 3- Vinegar (5%) used directly without water dilution.

### *Microbiological evaluation*

The aerobic plate count, coliform count, and *Staphylococcus* count were measured. Twelve samples from each group were examined microbiologically. The samples were prepared for microbiological evaluation following ISO 18593: [20]. The samples were placed immediately in 1 ml of 0.1% solution of peptone broth. After that, they were kept at 4°C until plating was performed. Then, the broth was diluted for subsequent microbial measurements. The procedures for microbiological measurements followed Foods [21].

### *Aerobic plate count "APC"*

For APC, the pour-plate method was used. We inoculated 1 ml of the previously prepared serial dilutions into the agar. Then, the plates were incubated for 24 to 48 hours at 35°C. A colony counter was used for counting plates containing 30 and 300 colonies. The total APC was obtained by multiplying the total number of colonies by the dilution factor.

### *Coliform count "CC"*

The CC was determined via the pour-plate method. We added 1 ml of the previously prepared serial dilutions to melted violet-red bile (VRBA) agar. After that, the plates were incubated at 37°C for 24 hours. Then, the plates were kept at 35°C for 24 to 48 hours. A colony counter was used for counting plates containing 30 and 300 colonies. We counted the colonies that showed a purple circle around them. The total CC was obtained by multiplying the total number of colonies by the dilution factor.

### *Staphylococci count "SC"*

*Staph. aureus* colonies were counted on Baird-Parker agar plates. We used 0.1 ml of the serial dilutions that had been made earlier for inoculation. Then, the plates were incubated for 24 to 48 hours at 35±2°C. Round, shiny, smooth, convex, and black colonies were counted.

### *Histology*

Half a centimeter-long meat samples from each group were preserved in 10% neutral buffered formalin for one week. Then, the samples were dehydrated with ascending grades of ethyl alcohol. Then, the samples were cleared via xylene and stained with hematoxylin and eosin for further histological observation according to Bancroft and Gamble [22]. Images were acquired with a Leica DM 3000 light microscope.

### *Sensory Evaluation and Overall Acceptance*

Each sample was evaluated by 9 well-trained panelists. Everyone served a turkey sample (100 ± 10 g) for each vinegar concentration. The panelists were asked to evaluate the sensory qualities (color, odor, texture, and taste). The samples were coded with random numbers; the panelists were not acquainted with the experimental approach. They were requested to give a score indicating the overall acceptance of each sample. A nine-point descriptive scale was used; a score of 9 was the highest, while a score of 1 was the lowest according to Civille and Carr [23].

### *Statistical analysis*

A logarithmic transformation of the obtained results was then performed using a paired samples t test in SPSS according to Feldman, Ganon [24]. The results of the bacterial counts (log CFU/g) are

expressed as the standard error of deviation (SD).  $P \leq 0.05$  was considered to indicate a significant difference.

## Results

### Microbiological evaluation

The application of vinegar dipping resulted in considerable reductions in the evaluated bacteriological parameters and a strong antimicrobial effect, as indicated in Tables 1, 2, and 3. Increases in vinegar concentration were associated with greater reductions in microbial counts.

#### Total aerobic plate count (APC)

The mean APC count in the control group was 4.52 log CFU/g. This value was significantly ( $p \leq 0.01$ ) greater than that of the vinegar-treated samples. The mean APC count was 3.08 log CFU/g in G2, 1.78 log CFU/g in G3 and 0.18 log CFU/g in G4. The reduction percentages (R%) were 32.18, 60.48, and 94.54 in G2, G3, and G4, respectively. The data are shown in Table (1).

#### Total coliform count (CC)

The mean CC in the control group was 3.37 logs CFU/g. This value was significantly ( $p \leq 0.01$ ) greater than that of the vinegar-treated samples. The mean CC value was 2.13 log CFU/g in G2, 1.29 log CFU/g in G3 and 0.18 log CFU/g in G4. The R% was 37.08, 61.63, and 94.54 in G2, G3, and G4, respectively. The data are shown in Table (2).

#### *Staphylococcus aureus* (*Staph. aureus*) count

The mean *staph. aureus* in the control group 2.9 logs CFU/g. This value was significantly ( $p \leq 0.01$ ) greater than that of the vinegar-treated samples. The average values were 1.8 log CFU/g in G2, 0.51 log CFU/g in G3 and 0.00 log CFU/g in G4. The R% was 37.71, 82.91, and 100 for G2, G3, and G4, respectively. The data are shown in Table (3).

Therefore, the suppression of microbial growth was shown to be proportional to the vinegar content. The use of undiluted vinegar with an initial acidity of 5% was the best way to decontaminate turkey meat. We noticed that *Staph. aureus* was the most sensitive bacterium to vinegar acidity.

### Histology

Histological observation confirmed the efficiency of vinegar in preserving muscle structure (Fig. 1). The absence of skeletal muscle striations was detected in some muscle fibers of the control group (Fig. 1A). However, dipping muscles with various dilutions of vinegar improved muscle striations (Fig. 1B-D). Dipping of muscles for 20 minutes in undiluted vinegar (5%) in the G4 group resulted in

better preservation of muscle structure and improved cellular staining (Fig. 1D).

### Sensory Evaluation and Overall Acceptance

The turkey breast meat samples were sensory evaluated in the four experimental groups, as shown in Table (4). The samples in the control group (G1) were excellent in color and odor and very good in texture. The samples in G2 were very good in color, odor, and texture. The samples in G3 were very good in color and odor and very good in texture. However, the samples in G4 were very good in color and odor and excellent in texture. However, for taste evaluation, G1 and G2 exhibited very good scores, G3 had very good scores, and G4 exhibited excellent scores. Therefore, the texture and taste of the vinegar-treated turkey meat were better than those of the control group.

## Discussion

The food sector is keenly interested in developing natural preservative alternatives to synthetic alternatives, as the World Health Organization acknowledged in 2015 that certain foods containing chemical preservatives can cause cancer [25]. Therefore, the use of natural preservatives rather than artificial preservatives has received increased attention [26]. Turkey meat contamination typically occurs as a result of improper dressing, handling, transportation, storage, and slaughtering practices [27]. Our previous microbiological evaluation of frozen turkey breast and thigh meat indicated the presence of foodborne pathogens. The APC, CC, SC, mold and yeast count, *Escherichia coli* (*E. coli*) incidence, and *Salmonella* incidence were indicated in both the thigh and breast of frozen turkey meat. The thigh meat exhibited greater bacterial contamination than the breast meat [28]. Therefore, in this study, we attempted to identify a proper technique for the decontamination of frozen turkey meat from supermarkets in Egypt. This study is important for preventing food-borne illnesses and maintaining the health of human consumers.

Although several techniques have been used to reduce microbial contamination of meat, it is difficult to completely prevent food-borne infections. One antimicrobial technique that is used to decontaminate turkey meat is organic acid (vinegar) dipping. This technique significantly reduces the amount of pathogenic bacteria in beef meat, particularly coliforms, *staphylococci*, and other aerobic pathogens that cause food spoilage [29]. In addition, it was also shown to prevent spore germination and outgrowth of *Clostridium perfringens* bacteria in turkey meat [30].

Jay, Loessner [31] previously reported that organic acids, particularly acetic and lactic acids, were applied to the whole surface of a carcass as warm showers. Based on our results, it seemed that

the vinegar employed had a high potential antibacterial effect, particularly when the concentration of the vinegar utilized increased. This outcome is consistent with the findings of [32, 33], who reported that acetic acid is the most effective organic acid for removing bacteria from sheep carcasses overall. Greater acid concentrations achieved better decontamination than lower concentrations.

The vinegar dilution and the time the meat was dipped affected the microbiological quality of the meat. [34] reported that applying commercial vinegar containing 5% acetic acid (pH 3.0) for five minutes to inoculated lettuce ( $10^7$  CFU g<sup>-1</sup>) resulted in a three-log reduction in the microbial population at 25°C. Five minutes is not sufficient time for complete microbial decontamination. Therefore, in this study, we increased the time to 20 minutes to determine the viability of vinegar for meat decontamination at room temperature. Our results confirmed that vinegar containing 5% acetic acid efficiently promoted meat decontamination after 20 minutes at room temperature.

Vinegar was proven to be an effective natural preservative against total aerobic bacteria, *Pseudomonas* spp., and lactic acid bacteria. It can extend the shelf life of hummus (a Mediterranean ready-to-eat food) when it is stored at 4°C for 21 days [35]. In addition, vinegar was proven to be suitable for short-term storage of beef meat in a refrigerator (4±1°C) [15]. However, little is known about the availability of vinegar for turkey meat decontamination at room temperature.

Histological observation of meat marinated with weak organic acids and NaCl revealed distinct changes in collagen fibers and muscle fiber structure [36]. However, little is known about the effect of vinegar consumption on turkey meat.

Our results showed that the best sensory quality was achieved in turkey breast meat samples treated with 5% vinegar. Sensory evaluation is a quick, efficient, and easy method for obtaining information about the acceptance and overall quality of a product. It depends on organoleptic characteristics such as color, odor, texture, and overall acceptability of the product [37]. Following our findings, high vinegar concentrations had a potent antibacterial effect, although no adverse organoleptic changes were noted [38]. Sarker, Hashem [15] also reported that the addition of different concentrations of vinegar significantly improved the microbiological, sensory, and other physicochemical properties of vinegar-treated meat.

### **Conclusion**

Frozen turkey meat samples that had been immersed in 1, 3, or 5% vinegar for 20 minutes exhibited an obvious reduction in the number of microorganisms. Dipping turkey meat in 5% vinegar without water dilution for 20 minutes at room temperature was superior. Therefore, we recommend washing frozen turkey meat with vinegar (acidity, 5%) for 20 minutes at room temperature before cooking it for optimum meat quality.

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Not applicable.

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This study didn't receive any funding support

### **Conflict of Interest**

The authors declare that there is no conflict of interest.

### **Ethics approval**

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt (ethics approval number; 49/11/2023).

**TABLE 1. Statistical analysis of Aerobic plate count (log CFU/g) in the examined samples of turkey breast (n=12)**

Samples/ groups	Min ± SD (log CFU/g)	Max ± SD (log CFU/g)	Mean ± SD (log CFU/g)	R%
Group 1 (control)	4.07 <sup>a</sup> ± 0.2	4.82 <sup>a</sup> ± 0.11	4.52 ± 0.12	zero
Group 2	2.92 <sup>b</sup> ± 0.14	3.25 <sup>b</sup> ± 0.12	3.08 ± 0.12	32.18
Group 3	1.22 <sup>b</sup> ± 0.12	2.07 <sup>b</sup> ± 0.07	1.78 ± 0.10	60.48
Group 4	ND*	1.11 <sup>b</sup> ± 0.01	0.18 ± 0.04	94.54

ND\*= Not detected, R= Reduction, \*Reduction % = (control –treated)/ control \*100, and different superscript letters in the same rows are significantly different at (P≤0.05).

**TABLE 2. Statistical analysis of Coliform count (log CFU/g) in the examined samples of turkey breast (n=12)**

Samples/ groups	Min $\pm$ SD (log CFU/g)	Max $\pm$ SD (log CFU/g)	Mean $\pm$ SD (log CFU/g)	R%
Group 1 (control)	3.07 <sup>a</sup> $\pm$ 0.14	3.75 <sup>a</sup> $\pm$ 0.04	3.37 $\pm$ 0.08	zero
Group 2	2.01 <sup>b</sup> $\pm$ 0.08	2.41 <sup>b</sup> $\pm$ 0.02	2.13 $\pm$ 0.07	37.08
Group 3	1.13 <sup>b</sup> $\pm$ 0.11	1.81 <sup>b</sup> $\pm$ 0.02	1.29 $\pm$ 0.08	61.63
Group 4	ND*	1.11 <sup>b</sup> $\pm$ 0.01	0.18 $\pm$ 0.00	94.54

ND\*= Not detected, R= Reduction, \*Reduction % = (control –treated)/ control \*100, and different superscript letters in the same rows are significantly different at (P $\leq$ 0.05).

**TABLE 3. Statistical analysis of *Staphylococcus aureus* count (log CFU/g) in the examined samples of turkey breast (n=12)**

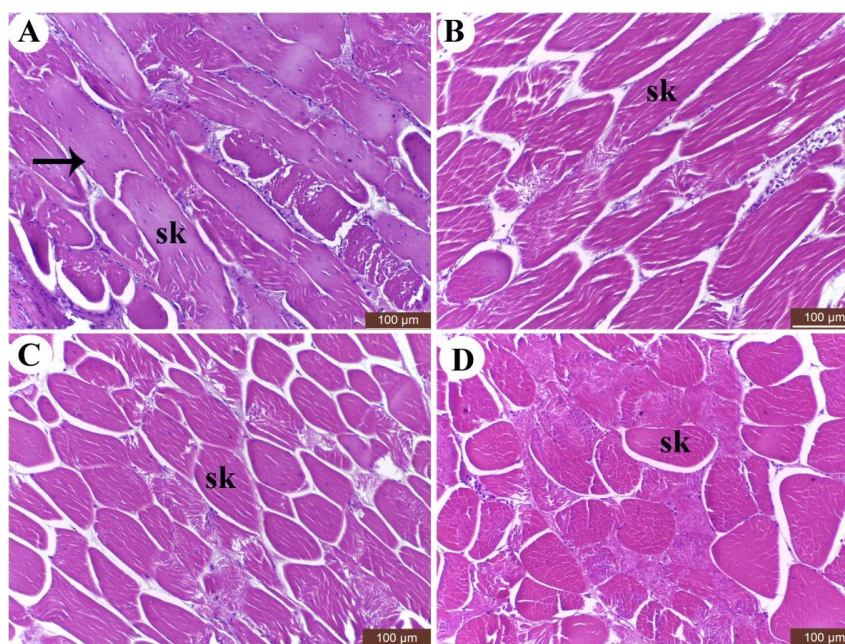
Samples/ groups	Min $\pm$ SD (log CFU/g)	Max $\pm$ SD (log CFU/g)	Mean $\pm$ SD (log CFU/g)	R%
Group 1 (control)	2.54 <sup>a</sup> $\pm$ 0.11	3.21 <sup>a</sup> $\pm$ 0.15	2.9 $\pm$ 0.09	zero
Group 2	1.54 <sup>b</sup> $\pm$ 0.11	2.11 <sup>b</sup> $\pm$ 0.09	1.8 $\pm$ 0.07	37.71
Group 3	ND*	1.07 <sup>b</sup> $\pm$ 0.02	0.51 $\pm$ 0.01	82.91
Group 4	ND*	ND*	Zero $\pm$ 0.00	100

ND\*= Not detected, R= Reduction, \*Reduction % = (control –treated)/ control \*100, and different superscript letters in the same rows are significantly different at (P $\leq$ 0.05).

**TABLE 4. A pattern of overall acceptance (color, odor, texture, and taste) of fresh turkey breast meat samples treated with three different vinegar acidity concentrations.**

Samples/ groups	Color	Odor	Texture	Taste
Group 1 (control)	9.00 $\pm$ 0.00	9.00 $\pm$ 0.00	7.33 $\pm$ 0.33	7.00 $\pm$ 0.00
Group 2	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	8.00 $\pm$ 0.00	7.33 $\pm$ 0.00
Group 3	7.33 $\pm$ 0.00	7.33 $\pm$ 0.00	8.33 $\pm$ 0.33	8.00 $\pm$ 0.00
Group 4	7.00 $\pm$ 0.00	7.00 $\pm$ 0.00	9.00 $\pm$ 0.00	9.00 $\pm$ 0.00

Score system: 9=Excellent, 8=Very very good, 7=Very good, 6=Good, 5 = Medium, 4 = Fair, 3 = Poor, 2=Very poor, and 1=Very very poor



**Fig. 1. Histological observation of Turkey's breast muscle. (A) Control group, without the addition of vinegar. (B) Vinegar was added at 1% acidity dilution. (C) Vinegar was added at 3% acidity dilution. (D) Vinegar was added at 5% acidity dilution. Note, the absence of striations (arrow) in the skeletal muscles (sk) of the control group. While, obvious striations in the skeletal muscles (sk) after the addition of vinegar are noticed (B, C, and D).**

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## كفاءة الخل في تحسين الجودة الميكروبيولوجية والنسجية للحوم الديك الرومي المجمدة

رانيا عاطف الخولي<sup>1</sup>، مني نصر عبدالنعم حسين<sup>2\*</sup>، نهلة أحمد شوقي أبو الروس<sup>3</sup>  
و فهيم عزيز الدين محمد شلتوت<sup>1</sup>

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<sup>2</sup> قسم الأنسجة والخلايا ، كلية الطب البيطري ، جامعة بنها ، مصر.

<sup>3</sup> قسم مراقبة الأغذية ، مركز بحوث صحة الحيوان ، مركز البحوث الزراعية فرع شبين الكوم ، مصر.

### الملخص

مسببات الأمراض المنقولة بالغذاء تهدد صحة الإنسان. ولذلك، أصبح من الضروري اتباع أساليب مناسبة للوقاية من الأمراض المنقولة بالغذاء وتحسين صحة المستهلكين من البشر. في هذه الدراسة تم تقييم قدرة الخل على تحسين جودة اللحوم. تم غمس لحم الديك الرومي لمدة 20 دقيقة في تخفيفات الحموضة 0 و 1 و 3 و 5% بالخل. تم بعد ذلك العدد الكلي للبكتيريا الهوائية (APC)، وعدد القولونيات (CC)، وعدد المكورات العنقودية (SC) لعينات اللحوم. تم إجراء التقييم الحسي والملاحظة النسيجية للألياف العضلية. كان العدد الكلي للبكتيريا الهوائية يساوي  $4.52 \pm 0.12 \log \text{cfu/g}$  و  $3.08 \pm 0.12 \log \text{cfu/g}$  و  $1.78 \pm 0.10 \log \text{cfu/g}$  و  $0.18 \pm 0.04 \log \text{cfu/g}$  لتخفيفات الحموضة 0 و 1 و 3 و 5% من الخل ، على التوالي. و عدد القولونيات كان يساوي  $3.37 \pm 0.08 \log \text{cfu/g}$  و  $2.13 \pm 0.07 \log \text{cfu/g}$  و  $1.29 \pm 0.08 \log \text{cfu/g}$  و  $0.18 \pm 0.00 \log \text{cfu/g}$  لتخفيفات الحموضة 0 و 1 و 3 و 5% من الخل ، على التوالي. وكان عدد المكورات العنقودية يساوي  $2.9 \pm 0.09 \log \text{cfu/g}$  و  $1.8 \pm 0.07 \log \text{cfu/g}$  و  $0.51 \pm 0.01 \log \text{cfu/g}$  و  $0 \pm 0.0 \log \text{cfu/g}$  لتخفيفات الحموضة 0 و 1 و 3 و 5% من الخل ، على التوالي. ومن الناحية النسيجية، تم الحفاظ على خطوط العضلات الهيكلية ، خاصة بعد غمس اللحم في الخل بنسبة 5%. بالإضافة إلى ذلك، أكد التقييم الحسي قدرة الخل على تحسين قوام ومذاق لحم الديك الرومي. وبناء على النتائج التي توصلنا إليها، ينصح بزيادة سلامة لحم الديك الرومي عن طريق غمسه في خل 5% لمدة 20 دقيقة. وبالتالي، فإن الخل هو وسيلة مباشرة واقتصادية وأمنة وفعالة للغاية لإزالة التلوث من اللحوم.

**الكلمات الدالة:** لحوم الطيور، العدد الكلي للبكتيريا ، المكورات العنقودية ، القولونيات ، إزالة التلوث.