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Original scientific paper

Effect of pomegranate (*Punica granatum*) fruit molasses as a natural marinade on the microbiological quality and shelf life of refrigerated chicken fillet

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ABSTRACT

Pomegranate molasses (PM) could be suitable as a marination ingredient in Mediterranean cuisine. This study is, thus, aimed at investigating the effects of PM on the microbial characteristics of chicken breast fillets. For this purpose, PM marinades were prepared at three different concentrations for the marination of chicken breast fillets, which were assigned to three treatment groups: T1 (immersed in 0.5% v/w PM), T2 (immersed in 1.0% v/w PM) and T3 (immersed in 1.5% v/w PM). Chicken breast fillets were marinated for 2 hours and then aerobically stored at 4°C for 15 days. Non-marinated fillets were used as the control. Levels of aerobic bacteria, psychrotrophic bacteria, coliforms, and lactic acid bacteria were determined to evaluate the evolution of spoilage. The results revealed that the growth rate of the microbial populations during storage at 4°C decreased with the increasing concentration of PM. The groups of aerobic, psychrotropic, and lactic acid bacteria may be continuously increased on each sampling day, with bacteria numbers on the control and T1 fillets surpassing those on fillets exposed to the other treatments ($p < 0.05$) from day 3 until day 15, when sampling stopped. All PM treatments had significantly decreased coliform counts ($p < 0.05$) than did the control group. At 4°C, the shelf life of PM-marinated chicken breast fillets was significantly extended compared to the controls, achieving up to 6, 9, and 12 days for T1, T2, and T3, respectively, as evaluated by microbiological analyses. The findings of this study suggest that pomegranate molasses could be used as an ingredient to improve the microbiocidal quality of marinade or as a sole marinade, both of which uses could prolong the shelf life of chicken breast fillets.

1. Introduction

Chicken meat is considered a desirable nutrient source for human health as it contains many polyunsaturated fatty acids, low lipid levels, and minerals. Also, it is low cost and has favorable organoleptic attributes, resulting in increased production and consumption of chicken meat in recent decades (*Zhang et al.*, 2021). However, chicken meat has a limited shelf life depending on various factors such as pre-slaughter handling, initial bacterial load, processing technology, pH, chemical composition and water activity of muscle, residual blood, carcass temperature, and storage and transport conditions (*Özünlü & Ergezer*, 2022).

In recent years, there has been growing demand for the use of natural additives and preservatives, rather than synthetic ones, in the food industry, as it has been claimed that synthetic additives and preservatives have hazardous and cancerous effects on human

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health (*Lemay et al*., 2002). Because of consumer awareness of the hazards of synthetic additives, people seek out foods devoid of them, increasing demand for natural preservatives that perform the same function as synthetic ones (*Kaderides et al*., 2021).

Pomegranate (*Punica granatum*) is cultivated in a wide range of areas, such as in the Mediterranean and South Asian regions. In recent years, pomegranate fruit production and consumption have increased due to the fruit's healthy beneficial effects, as it is used in treating inflammatory and digestive diseases (*El-Said et al*., 2014). Pomegranate molasses is derived from pomegranate juice, which is rich in total phenolic and flavonoid compounds and has become one of the most important additives that is becoming more applicable in different foods and widely distributed in various international markets in all countries, especially in the Middle East (*Nasser et al.*, 2017). There is increasing consumer interest in the nutritional value of pomegranate molasses, as it contains bioactive compounds, such as anthocyanins, ellagic acid derivatives, hydrolyzable tannins, and other phenolic compounds, that have anti-inflammatory and anti-tumor effects. Also, it gives the food a sweet taste and flavor (*Faour-Klingbeil & Todd*, 2018).

To the best of our knowledge, there are limited studies and literature on the role of pomegranate molasses in improving chicken meat shelf life, unlike other pomegranate by-products such as peel extract or juice. Therefore, this study was designed to examine the effect of marinating chicken breast muscle fillets in traditional pomegranate molasses in its pure form and at different concentrations; microbial levels were measured at three-day intervals and for the shelf life of the chicken breast fillets.

2. Materials and methods

2.1. Ethical approval

The study was conducted after the research proposal was approved by the Care and Use Committee Research Ethics, Faculty of Veterinary Medicine, Benha University (BUFVTM, 15/10/23), Egypt.

2.2. Preparation of pomegranate molasses

High-purity pomegranate molasses was purchased from the local market of El-Gharbia governorate, Egypt, in July 2022 and diluted in water to reach concentrations of 0.5%, 1% and 1.5%.

2.3. Chicken fillet treatment and storage

Chicken fillets were purchased from the local market of El-Gharbia governorate, Egypt and transferred to the laboratory in cold conditions. The chicken fillets were divided into four parts and transferred into four containers. To marinate fillets, they were soaked in a pomegranate molasses/water solution containing 0% (CO), 0.5% (T1), 1% (T2), or 1.5% (T3) molasses for two hours. The marinated fillets were placed in sterile bags and kept at 4°C, and fillet microbiological evaluation was conducted at three-day intervals.

2.4. Microbiological Analysis

2.4.1. Sample preparation

The chicken breast fillet samples for microbiological analyses were prepared by placing 10 g of excised fillet into 90 ml of peptone water (0.1%), mixing in a sterile bag, and homogenizing with a Stomacher (Stomacher® 400 Circulator, Seward, UK) at 200 rpm/min for 1 minute. Then, other decimal dilutions were prepared from this dilution in tubes containing peptone water.

2.4.2. Microbiological analysis

Bacterial counts were performed using plate count agar (C# CM0325, OXOID, UK) for aerobic plate count (APC); the inoculated plates were incubated at 30±1 °C for 72±3 h (ISO, 2013). The psychotropic plate count (PPC) was performed using plate count agar (C# CM0325, OXOID, UK), and the inoculated plates were incubated at 7 °C for 10 days (ISO, 2019). The lactic acid bacteria count (LAB) was performed using De Man-Rogosa Sharp Agar (MRS) (C# NCM0190A, NEOGEN®, USA). The incubation conditions were 30±1 °C for 72±3 h for LAB (ISO, 1998). Violet red bile agar (VRBA) (C# CM0485, Oxoid, UK) plates were used for total coliform count (TCC), and the inoculated plates were incubated at 30±1 °C for 72±3 h (ISO, 2006). All counts were expressed as $\log_{10}$CFU/g and were performed in duplicate.

2.5. Microbial reduction calculation

Using the average values of these counts, the microbial reduction percentages were calculated according to the following formula in relation to the control: Microbial reduction percentage (%) = (control CFU − test CFU)/control CFU) × 100.

In addition, the logarithmic scale reduction factor (log_{10}) was calculated using the formula RF = Log_{10} (A) − Log_{10} (B), where A is the number of colonies recovered from the unexposed (control) and B is the number of colonies recovered from the exposed (test) from T1 to T3 (*Mascarenhas et al.*, 2022).

2.6. Statistical Analyses

The collected data were exposed to one-way Analysis of Variance (ANOVA) using SPSS (version 20; IBM, Chicago, IL, USA) followed by Tukey's multiple comparison tests (Tukey, 1953) to compare the differences between dietary treatments, where significant differences were observed ($p \leq 0.05$).

3. Results

The effects of marinating chicken breast meat fillets in PM at different concentrations and storing them at refrigeration temperature were studied. To assess the progression of spoilage and shelf-life extension, APC, PPC, TCC, and LAB counts of the chicken breast meat fillets were measured. (Figures 1–4).

The initial APC in all treated chicken fillet groups ranged from 4.7 to 4.3 log_{10}CFU/g at the beginning of the storage period. During the 4°C storage period, the APCs of control and PM-treated chicken fillet groups increased. The control fillet group reached the limit of acceptability on day 6, as the APC was 6.02 log_{10}CFU/g, which is the point that indicates the spoilage of chicken meat (red dash-dot line in Figure 1A). However, APCs in the treated fillets increased more slowly than those in the control fillets. As the PM concentration increased, there was a significant slowdown in the growth and a relative reduction of the APC ($p < 0.05$). T3 had the greatest reduction in APC compared to the control, as it exceeded the spoilage limit of 6.5 log_{10}CFU/g after 15 days of storage (Figure 1A). The mean APC reduction percent due to marinating fillets in PM was significantly lower ($p<0.05$) than the control. This reduction trend continued on days 6, 9, 12, and even day 15, as T3 APC reduction percent was significantly ($p < 0.05$) greater than any other treatments at those times (Figure 1B).

The PPC bacteria increased in all fillet groups during the period of storage. The maximum PPC increase was recorded in the control group, going from 4.5 log_{10}CFU/g at initial storage to 7.7 log_{10}CFU/g at final storage, increasing by 3.2 log cycles during the shelf life. On the other hand, the groups marinated in PM showed lower growth of PPC during the storage life as compared to the control, as recorded on day 15: 7.5 log_{10}CFU/g,7.2 log_{10}CFU/g, and 7.0 log_{10}CFU/g in T1, T2, and T3, respectively (Figure 2A). In this study, PPC on T3 fillets did not exceed 6 log_{10}CFU/g until the 12th day; this level was determined at the start of the study as the PPC threshold for non-consumable products (Figure 2A). The mean PPC reduction percent due to marinating fillets in PM was significantly greater ($p < 0.05$) than in the control. This reduction pattern began on day 0 and continued through sampling on days 6, 9, 12, and 15, as T3 PPC reduction percent was substantially ($p < 0.05$) greater than reductions for any other treatment on those days,

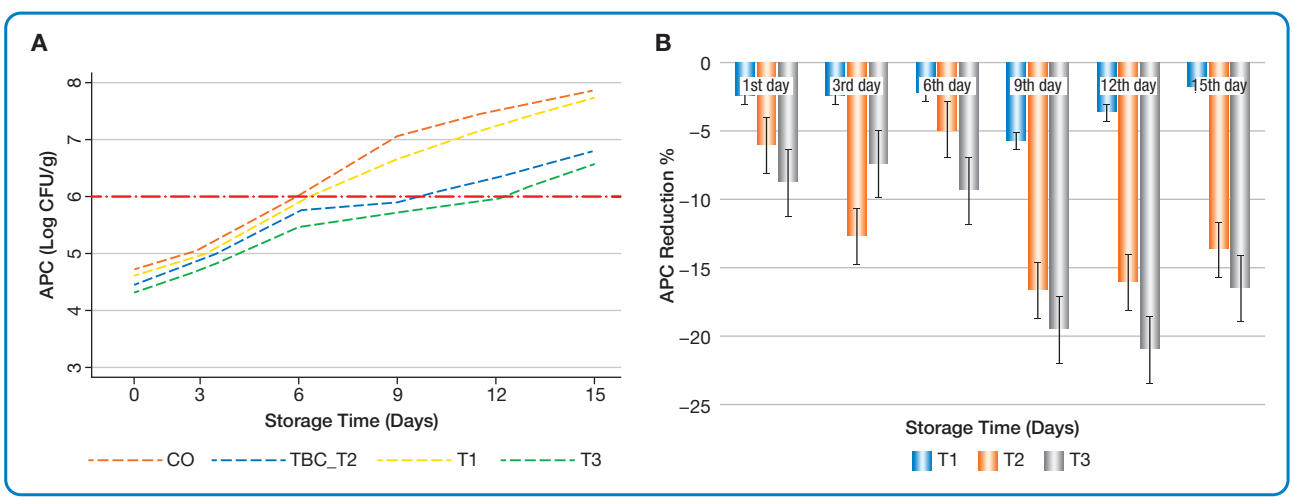

Figure 1. (A): Evolution of APC (log_{10} CFU/g) counts of control (CO) and pomegranate molasses-marinated chicken breast fillet groups; T1 (0.5% v/w), T2 (1% v/w) and T3 (1.5% v/w), stored at 4 °C, (B) Microbial reduction percentage of APC

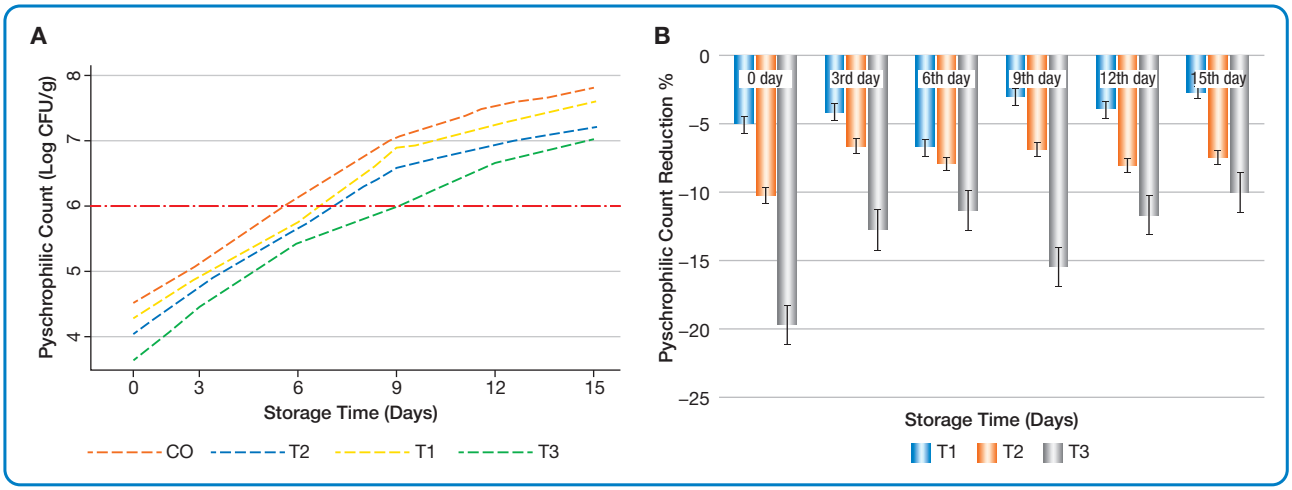

Figure 2. (A): Evolution of PPC (log_{10} CFU/g) counts of control (CO) and pomegranate molasses-marinated chicken breast fillet groups; T1 (0.5% v/w), T2 (1% v/w) and T3 (1.5% v/w) stored at 4 °C, (B): Microbial reduction percentage of PPC

with the best reduction in T3 recorded on day 0 and generally declining with time (Figure 2B).

The LAB counts on day 0 ranged from 3.8 log_{10} CFU/g in T3 to 4.5 log_{10} CFU/g in the control group. During the storage period, the growth of LAB was greatest on the control chicken fillet group (LAB numbers reached 7.5 log_{10} CFU/g) followed by T1 (7.2 log_{10} CFU/g), then T2 (6.4 log_{10} CFU/g), and T3 (6.0 log_{10} CFU/g) on day 15 of storage (Figure 3A). The control chicken fillets exceeded the deterioration limit (6 log_{10} CFU/g) on day 6, while the T2 and T3 groups exceeded the limit in 12 and 15 days, respectively. T3 group's LAB reduction percent was substantially ($p < 0.05$) greater than seen in any other treatment group on the same respective days, with the best reduction seen on day 15 (Figure 3B).

Results of the TCC microbial analysis are presented in Figure 4. The TCC was countable on day 0 in the control fillet group, being 2.6 log_{10} CFU/g, but TTCs remained below the limit of detection in the other treatment groups until day 3 of storage (Figure 4A). Throughout the storage, the TCC in the control group increased until it reached 5.6 log_{10} CFU/g at the end of storage. In contrast, the TCC in the PM-treated chicken fillets began to be detected from day 3 in the T1 and T2 fillet groups, with counts of 2.8 log_{10} CFU/g and 2.6 log_{10} CFU/g, respectively, until TCC reached around 5 log_{10} CFU/g in both groups after 15 days of storage. In the T3 fillet group, the TCC remained below the limit of detection until day 6, when the count was 2.6 log_{10} CFU/g, and increased to 4.3 log_{10} CFU/g at the end of storage (Figure 4A). T3 fillet group's TCC

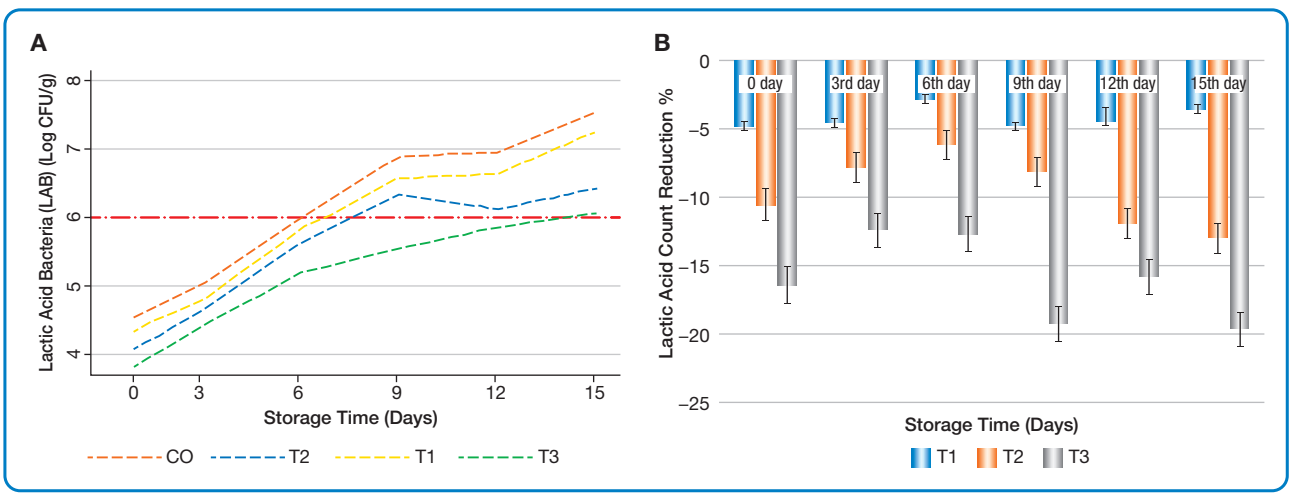

Figure 3. (A): Evolution of LAB (log_{10} CFU/g) counts of control (CO) and pomegranate molasses-marinated chicken breast fillet groups; T1 (0.5% v/w), T2 (1% v/w) and T3 (1.5% v/w) stored at 4 °C, (B): Microbial reduction percentage of LAB

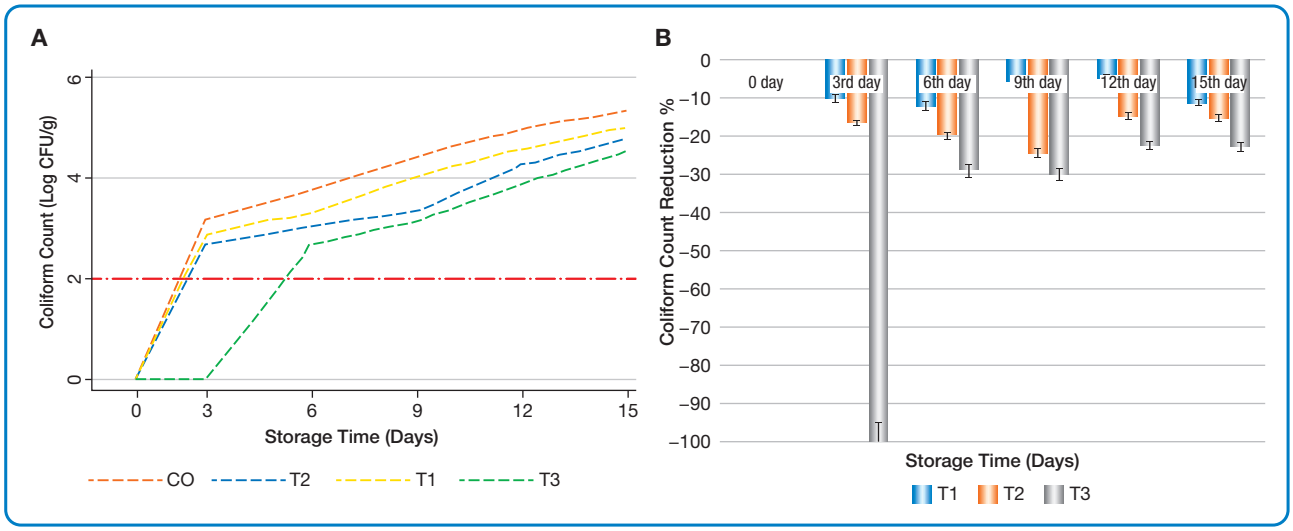

Figure 4. Evolution of TCC (log_{10} CFU/g) counts of control (CO) and pomegranate molasses-marinated chicken breast fillet groups; T1 (0.5% v/w), T2 (1% v/w) and T3 (1.5% v/w) stored at 4°C

reduction percentage was significantly ($p < 0.05$) greater than in any other fillet group on the same respective day, with the greatest reduction on day 3 (Figure 4B).

4. Discussion

Bacteria are one of the main agents that cause fresh meat to lose its quality, as these products are more likely to be spoiled with microorganisms if they are not properly handled and preserved in good condition; therefore, nowadays, there is an increase in the use of preservatives with antimicrobial properties (*Pires et al*., 2022). Microbiological assessment, including bacterial determination, is an important key in evaluating the safety and quality of marinated chicken meat, as it is quick, inexpensive, and very accurate in identifying bacteria (*Augustyńska-Prejsnar et al*., 2023).

Pomegranate fruit has polyphenolic compounds that inhibit the microbial growth that is responsible for food deterioration and food-borne illness (*Çam et al.*, 2014). Many phytochemical compounds in pomegranate have antimicrobial activity, such as ellagic acid and larger hydrolyzable tannins, such as punicalagin (*Ferrazzano et al*., 2017). The pomegranate fruit and its derivatives possess antibacterial activity against both Gram-negative and -positive bacteria due to the broad spectrum of bioactive compounds with antimicrobial characteristics (like polyphenol, ellagic and tannins) in the fruit (*Gullon et al*., 2016). In this study, PM at high concentrations in marinade was found to be able to lengthen the shelf life of the chicken fillets, agreeing with (*Zhuang et al*., 2019a), who said that treating big head carp fillet with pomegranate peel extract increased the shelf life by 1–2 days over that of the control. The shelf life of chicken meat can be determined by the total aerobic count, with the recommended limit in chilled chicken meat being 6 log_{10} CFU/g (NFSA, 2021). In this study, the APC of the control group was 4.7 log_{10} CFU/g initially, which agrees with other results (*Bazargani-Gilani et al*., 2015a; *Vaithiyanathan et al*., 2011), which reported initial APCs were 4.5 log_{10} CFU/g and 4.8 log_{10} CFU/g for chicken meat. Microbial increases in chicken meat during storage at refrigerated temperature result from deterioration of the physicochemical characteristics of meat (*Sujiwo et al*., 2018).

The APC in our T3 group was 6.5 log_{10} CFU/g at the end of storage; this agreed with *Rahnemoon et al*. (2021), who observed that their coated chicken meat with nano-encapsulated pomegranate peel extract reached the same limit after 14 days. Also, the APCs in shrimp dipped in 1 g/100 mL pomegranate extract (5.99 log_{10} CFU/g) and 2 g/100 mL pomegranate extract (5.96 log_{10} CFU/g) were lower than in the control (*Basiri et al.*, 2015). However, there was no significant difference between the treatment groups in that study. In addition, *Ghimire et al*. (2022) found a significant difference between APCs in the control and their pomegranate peel extract-incorporated ground buffalo meat. However, there was no significant difference in APCs between the 1% and 1.5% pomegranate peel extract-incorporated buffalo meat. Finally, pomegranate juice and extract had an antibacterial effect, confirmed by minimum APCs on frozen burgers (*Shahamirian et al*., 2019).

The current results revealed that our T2 and T3 chicken fillet groups exceeded the upper permissible limit for acceptability, which was an APC of 7.0 log_{10}

CFU/g, on days 12 and 15 of storage, respectively. We speculate this extension of the shelf life was due to the antimicrobial action of the condensed compound of pomegranate, especially tannins and protein perceptible compound (*Bazargani-Gilani et al*., 2015a). The APCs of chicken meat products enclosed with pomegranate rind extract increased during the storage life. However, APCs on the pomegranate-treated products were lower than those of the control on all sampling days (Bazargani-Gilani et al., 2015a), likely because of the phenolic compounds and other components of pomegranate rind extract, which are reported to have antimicrobial properties against many microorganisms in meat products (*Dua et al*., 2016). Pomegranate peel extract in buffalo meat has been reported to prolong the shelf life and the meat's quality for up to an 8-day storage period (*Rasuli et al*., 2021). In another study, the total aerobic count was significantly lower in Frankfurter containing pomegranate juice concentrate and rind powder than in the control, and this indicates the pomegranate juice concentrate and rind powder is a wealthy source of phytochemical and phenolic compounds that provide antibacterial activity against a wide range of microorganisms (*Firuzi et al*., 2019).

Psychrotrophic bacteria are the most prevalent bacteria on refrigerated chicken by-products. They are the microorganisms of choice to detect the true microbial loads of chicken products and provide better detection of issues regarding product temperature (*Cortez-Vega et al*., 2012). The current study's results agreed with *Özünlü and Ergezer* (2022), who stated that the psychrophilic counts gradually increased with storage time and product unacceptability for human consumption, while it is considered chicken breast meat is spoiled at a PPC level of 6.0 $\log_{10}$ CFU/g. The smaller increase of PPC we found in PM-marinated chicken fillets (compared with the control PPC) complied with the result obtained by *Bazargani-Gilani et al*. (2015b), who found that the highest count in chicken meat was recorded in the control group rather than in other treatments that contained pomegranate juice; the pomegranate phenolic compound inhibited the psychrotrophic bacteria under the chill storage conditions. Moreover, treatments with 1 and 2 g/100 mL pomegranate extract resulted in decreasing PPC in shrimps compared to the control (*Basiri et al*., 2015). The psychrotrophic and thermophilic counts of chicken meat patties treated with pomegranate by-products and their extracts were significantly lower than the control group's counts (*Sharma & Yadav*, 2020). Similarly, the PPC remained lower in the meat sample with pomegranate juice than in the untreated control from 14 to 28 days of storage (*Vaithiyanathan et al.*, 2011). Those authors reported that the significant concentration of detectable phenolic compounds and condensed tannins in pomegranate juice provided antibacterial action in samples treated with the juice through protein binding or enzyme inhibition (*Vaithiyanathan et al*., 2011).

The LAB is one group of multiple bacteria genera associated with the spoilage of chicken meat during the storage period (*Pellissery et al*., 2019; *Zhuang et al*., 2019b). In the current study, the LAB level on the fresh control was 3.8 $\log_{10}$ CFU/g on day 0, but 7.5 $\log_{10}$ CFU/g on the final storage day (day 15), which was slightly lower than the LAB counts reported by Bazargani-Gilani et al. (2015b) and (*Fratianni et al*., 2010). The slower increase of the LAB observed in this study on T2 and T3 groups (compared with the control and T1) was in line with the results of *Bazargani-Gilani et al.* (2015b) and *Basiri et al*. (2015), who reported that pomegranate juice has a lowering effect on the LAB on chicken compared to the control during storage. T3 produced the lowest LAB count, indicating that marinating chicken fillets in PM influences LAB growth, particularly as the concentration increases.

The low TCC level observed in this study was due to coliforms being not primarily present in the freshly slaughtered carcasses and having a lower count than mesophilic bacteria. The presence of coliforms indicates direct or indirect contamination with a high degree of contamination (*Fliss et al.*, 1991). In fact, coliforms on chicken carcasses can be connected with the absence of hygiene or sanitary conditions during slaughter and processing (*De Moura Oliveira et al*., 2005). Low counts of these microorganisms can indicate good sanitary conditions of the chicken breast meat (*Özünlü & Ergezer*, 2022). In our study, the absence of coliforms on PM-marinated chicken fillets in the initial storage stage could have been due to the low pH of pomegranate, considered an inhibitory factor that can limit the growth of bacteria. The direct bactericidal action of organic acids results from a pH decrease within bacterial cells (*Raftari et al*., 2009). The TCC on the control in our study was 2.6 $\log_{10}$ CFU/g on day 0, which was lower than reported by *Bhoir et al.*, (2019), who found that TCCs in untreated samples were 4.22 $\log_{10}$ CFU/g on day 0.

The slowing of coliform growth observed in this study with PM marination agreed with the result (*Dakheli*, 2020) when pomegranate waste extract caused significant minimization in the number of coliforms on poultry carcasses compared with the controls. The increase in the concentration of the pomegranate extract resulted in a significant decrease in coliforms in the treated poultry carcass groups *(Dakheli*, 2020). *Kanatt et al*. (2010) reported that fecal coliforms were

not detected in any chicken lollipop samples containing 0.1% and 0.5% pomegranate extract during 12 days of storage, and also reported that 1% pomegranate extract increased the shelf life of chicken by two weeks due to the antimicrobial action of phenolic compounds in the plant extract. Our study's results were also in agreement with *El-Nashi et al.* (2015), who reported that TCCs decreased during storage of beef sausages treated with different concentrations of pomegranate peel powder compared with control.

The polyphenolic compounds (flavonoids, tannins) from plant components like pomegranate fruit by-products have antibacterial properties. These secondary metabolites inhibit bacteria by forming complexes with proteins and sulfhydryl groups that make them unavailable for the microorganisms (*Indices et al*., 2021). The possible mechanism of the antimicrobial effect of the pomegranate extract might be related to their phenolic compounds, as these can bind to substrates such as minerals, vitamins, and carbohydrates, making them inaccessible to microorganisms; phenolic compounds can also denature enzymes. Furthermore, phenols can disturb the structure and function of the cell membrane (*Essid et al*., 2020).

5. Conclusion

In conclusion, marination in pomegranate molasses effectively delayed chicken breast fillet spoilage. Microbial analysis showed that pomegranate molasses, a natural product, can control the microbial growth in chicken breast muscle fillets marinated in pomegranate molasses (1.5% v/w) and stored under refrigeration at 4 °C. The treated breast meat fillets were microbiologically acceptable for 12 days of refrigerated storage. The limited microbial growth that occurred suggests that poultry meat processors could utilize these findings to replace chemical preservatives in ready-to-cook poultry products without lowering quality and shelf life, and without decreasing consumer acceptance of the products. Thus, using pomegranate-based marinades could lead to convenient and upgraded ready-to-cook products.

Uticaj voćne melase nara (*Punica granatum*) kao prirodne marinade na mikrobiološki kvalitet i rok trajanja rashlađenog pilećeg filea

Hanaa S. Bekeir, Ahmed Hamad, Nesreen Z. Eleiva i Reham A. Amin

INFORMACIJE O RADU

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APSTRAKT

Melasa od nara (PM) bi mogla biti pogodna kao sastojak za mariniranje u mediteranskoj kuhinji. Cilj ove studije je bio usmeren na istraživanje uticaja PM na mikrobne karakteristike fileta pilećih prsa. U tu svrhu pripremljene su PM marinade u tri različite koncentracije za mariniranje fileta pilećih prsa, koje su raspoređene u tri grupe tretmana: T1 (filei uronjeni u 0,5% v/w PM), T2 (filei uronjeni u 1,0% v/w PM) i T3 (filei uronjeni u 1,5% v/w PM). Fileti pilećih prsa su marinirani 2 sata, a zatim aerobno čuvani na 4 °C 15 dana. Kao kontrola korišćeni su nemarinirani fileti. Određeni su nivoi aerobnih bakterija, psihrotrofnih bakterija, koliformnih bakterija i bakterija mlečne kiseline da bi se procenila evolucija kvarenja. Rezultati su otkrili da se stopa rasta mikrobnih populacija tokom skladištenja na 4 °C smanjuje sa povećanjem koncentracije PM. Grupe aerobnih, psihrotropnih i bakterija mlečne kiseline mogu se kontinuirano povećavati svakog dana uzorkovanja, pri čemu broj bakterija u kontrolnoj grupi filea, kao i kod T1 grupe fileta nadmašuje one na filetima izloženim drugim tretmanima ($p < 0,05$), od 3. do 15. dana, kada je uzorkovanje prestalo. Svi tretmani PM su imali značajno smanjen broj koliformnih bakterija ($p < 0,05$) nego u kontrolnoj grupi. Na 4°C, rok trajanja fileta pilećih prsa mariniranih u PM je značajno produžen u poređenju sa kontrolom, dostižući do 6, 9, odnosno 12 dana za T1, T2 i T3, kako je procenjeno mikrobiološkim analizama. Nalazi ove studije sugerišu da bi melasa od nara mogla da se koristi kao sastojak za poboljšanje mikrobiocidnog kvaliteta marinade ili kao jedina marinada, a obe upotrebe bi mogle da produže rok trajanja fileta pilećih prsa.

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