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Research Article

Influence of Marinades on Oxidative Stability and Shelf Life of Marketed Spent Hen Meat

Rashid Ali Korejo[1], Atta Hussain Shah[1], Muneer Ahmed Jamali[1], Tarique Ahmed Khokhar[2]

[1]*Sindh Agriculture University Tandojam, Pakistan.*
[2]*Shaheed Benazir Bhutto University of Veterinary and Animal Sciences Sakrand, Pakistan.*

*Correspondence: vetdr_atta@sau.edu.pk

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Abstract

Lipid and protein oxidation occurs in meat during storage, that deteriorate the quality of meat, therefore the aim of study was to evaluate the impact of natural marinades on spent hen meat to reduce the oxidation and increase the shelf life of spent hen meat. The Meat sample was divided into seven groups i.e. control, yoghurt, ginger extract, tamarind juice, honey 20%, 25%, and 30% respectively. All groups were analyzed for thiobarbituric acid (TBA), free fatty acid (FFA), and peroxide value (POV) in lipid oxidation, for protein oxidation, tyrosine and sulfhydryl content. At intervals of 72 hours, the fresh TBA value (0.11) showed a significant difference (P≤0.05) with the control, tamarind juice and honey 30%. The FFA and POV showed non-significant (P≤0.05) deference with all treated groups, lowest value was observed in honey20%. The tyrosine value of fresh meat at 00 storage time was 0.65 in terms of protein oxidation; at 48 and 72 hour intervals, significant (P≤0.05) differences were noted with tamarind juice, honey 25%, and honey 30%, respectively. Fresh meat had a sulfhydryl content of 2.17, with exception of honey 20% at 48 hours of storage, showed significant (P≤0.05) difference with the control and all marinade groups at 48 and 72 hours interval. The TVC found in fresh meat was 6.12 to 10.31 at 00 to 72 hour storage while, marinades significantly affected the shelf life of spent hen meat. The fresh value coliform count (4.71) was statistically significant (P≤0.05) with all treated groups, whereas increasing trend was observed in all groups. The honey 20% showed the lowest results at 00 and 72 hours, respectively. It was observed that the marinades effectively reduce oxidation and extend shelf life.

Keywords: Oxidation; Peroxide value; Shelf-life; Spent hen; Thiobarbituric acid; Tyrosine value

Introduction

Poultry meat, an essential global energy and protein source, plays a crucial role in fulfilling the nutritional requirements of diverse populations. A mature female chicken

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that has completed her egg-laying cycle and is no longer economically viable for production is referred to as a "spent hen." Globally, billions of spent hens are produced each year, despite the possibility that some hens will make it to their second or third laying cycle (Jacob *et al.*, 2014). The utilization of spent hen meat holds notable significance in the poultry industry, not only for economic sustainability but also for maximizing resource efficiency (Zheng *et al.*, 2019). Despite their advanced age, spent hens represent a valuable meat source, emphasizing the importance of understanding how to improve its quality to promote sustainability and minimize waste. Spent hen meat is acknowledged for its nutritional richness, containing essential proteins, vitamins, and minerals that contribute to a well-rounded diet (Chen *et al.*, 2016), Despite that lipid oxidation and microbial development cause the product's quality attributes to deteriorate over storage. Reduced nutritional value and flavor changes are caused by lipid oxidation (Aguirrezábal *et al.*, 2000), whereas food poisoning and meat spoiling can result from microbial contamination and cause significant health risks as well as financial losses. According to Yin and Cheng (2003), applying appropriate agents with antimicrobial and antioxidant properties may help preserve meat quality, increase its shelf life, and avoid significant losses. Many artificial preservatives have been created, but most of them serve particular purposes in the meat system, such as inhibiting microbial growth or preventing lipid oxidation. Since many extracts or powders of herbs, plants, fruits, and vegetables have both antioxidant and antimicrobial properties, using natural preservatives to extend the shelf-life of meat products is a technique (Khare *et al.*, 2014).

Due to their perceived authenticity, natural agents with antibacterial and antioxidant capabilities have the benefit of being easily accepted by consumers (Sallam *et al.*, 2004). A number of commonly used spices and herbs are prized not only for their flavor and fragrance but also for their antimicrobial properties and therapeutic effects. In recent years, there has been a surge in interest in the use of plant extracts for a variety of pharmaceutical and food processing applications, and efforts to identify the bioactive principles of these extracts have made significant progress (Shan *et al.*, 2007).

The biological effects of polyphenolic compounds present in natural agents are diverse and include antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, cardioprotective, and vasodilatory properties (Benhammou *et al.*, 2009). Many processes, including free radical scavengers, reducing agents, pro-oxidant metal complexers, quenchers of singlet oxygen formation, and activators of antioxidative defense enzymes, have been linked to these functions and their antioxidant activity (Bertoncelj *et al.*, 2007).

Although the effects and mechanisms of protein oxidation in food systems are largely unknown, the role of oxidized proteins in the development of biological diseases has been studied for a few decades. The covalent modification of a protein caused by direct reactions with reactive oxygen species (ROS) or indirect reactions with secondary byproducts of oxidative stress is known as protein oxidation. The biological effects of elevated protein oxidation levels in humans and other animal species have been linked to a number of conditions, including aging and diseases (Zhang *et al.*, 2013). Because spent hen meat costs less than broiler meat, it is more affordable. But due to decline in the

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market for spent hen meat as the broiler industry, layer farms are finding it difficult to sell their chickens for a fair price. As consumers' preferences for natural foods grow, numerous studies are being conducted to decrease lipid oxidation and lengthen the shelf life of poultry meat by utilizing natural antioxidants. Marinades have antioxidant and antibacterial properties that improve the quality of meat. These characteristics eventually improve overall safety by preventing oxidative deterioration, lowering the risk of microbial contamination, and preserving the freshness of the meat. Therefore, the aim of the current study is to decrease the oxidation and increase the shelf life of meat by adding various natural marinades for utilization of spent hens.

Methodology

The spent hens were collected from Hyderabad market and underwent halal slaughtering procedure, then meat fillet was evaluated for the influence of marinades on oxidation and shelf life attributes of spent hen meat at the laboratory of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam for further analysis. To assess the impact of marinades on the oxidative and shelf life of spent hen meat, the meat samples were categorized into seven groups: i.e., Raw/control (group-1), marinade with yoghurt (group-2), ginger extract (group-3), tamarind Juice (group4), honey 20% (group-5), honey 25% (group-6), and honey 30% (group-7) with a fixed meat-to-marinade ratio "1:2 Meat: Marinade".

Marinades preparation

The ingredients for the marinades, which included yogurt made from milk, ginger, tamarind, and honey from the local market, were sourced locally and prepared in the laboratory. The yoghurt was made by fermentation. Ginger extract (GE) was prepared by peeling off, slicing the fresh ginger rhizome and blending with equal amount of distill water. Compressed tamarind was dissolved in distill water then all the seeds were discarded after stirring to prepare tamarind juice, and honey was mixed with distill water to form honey solutions at concentrations of 20%, 25%, and 30%. The specific composition of each marinade is detailed in table 1.

Table 1. Composition of various treatments.

S. No.	Marinade	Color	pH	Moisture %	Protein %	Fat %	Ash %	CF %	Carb%	Energy (kcal/100g)
1	Honey	Dark Brown	4.6	21.01	3.43	3.12	0.4	-	72.04	329.96
2	Yoghurt	Whitish	4.4	83.66	4.57	1.78	0.9	-	9.1	70.7
3	Ginger Extract	Pale Yellow	5.3	79.55	7.3	3.85	3.1	1.1	8.11	443.45
4	Tamarind Juice	Dark Black	5.7	51.14	6.7	3.31	1.9	2.7	34.25	319.59

Lipid oxidation

Thiobarbituric acid (tba) value

Thiobarbituric acid reagent was first prepared for TBA analysis. For this TBA reagent, 0.2883 g of thiobarbituric acid was dissolved in enough 90% acetic acid after the solution

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was slightly warmed. Then, 90 percent acetic acid is added to the volume to bring it to 100 ml. Then, 20 g of meat was blended for 2 minutes in 50 ml of cold 20% trichloro acetic acid to create trichloro acetic acid extract. The combined ingredients are mixed and filtered through a Whatman filter paper No.1after being rinsed with 50 ml of distilled water. Filtrate volume collected in a measuring cylinder with a 100 ml capacity. The filtrate is termed as trichloro acetic acid extract and is used in the estimation of TBA number. Trichloroacetic acid (TCA) extract and thiobarbituric acid (0.01 M) were blended in a volume of 5 ml each. After mixing, the test tube was immersed for 30 minutes in water bath. Along with a blank sample of 5 ml of 10% trichloroacetic acid was placed in a boiling water bath (100°C). The test tubes were taken out of the water bath after 30 minutes and allowed to cool for about 10 minutes while under running water. The amount of thiobarbituric acid in the meat was expressed as the developed color and measured as an absorbance value at 532 nm.

Peroxide value

In a 250 mL Erlenmeyer flask with a glass stopper, the samples (3g) were weighed. To melt the fat, it was then heated for three minutes at 60°C in a water bath. To dissolve the fat, the flask was then thoroughly stirred for 3 minutes with a 30 mL solution of acetic acid : chloroform (3:2 v/v). To remove meat particles from the filtrate, Whatman filter paper number 1 was used in the filtration process. After adding 0.5 mL of saturated potassium iodide solution to the filtrate, add starch solution as an indicator. The sodium thiosulfate standard solution was used to continue the titration. The following equation was used to calculate POV and express it as milli equivalents of peroxide per kilogram of sample:

$$\text{POV}\left(\frac{\text{meq}}{\text{kg}}\right) = \frac{(\text{S} \times \text{N})}{W} \times 100$$

Where “S” is the volume of titration (ml), “N” is the normality of sodium thiosulfate solution (N=0.01) and “W” is the sample weight (g).

Free fatty acid (ffa)

The sample (5g) was homogenized with 30 mL of chloroform at 10,000 rpm for one minute. To remove meat particles from the filtrate, Whatman filter paper number 1 was used in the filtration process. The titration was continued with solutions of 0.01 N ethanolic potassium hydroxide after the addition of five drops of 1% ethanolic phenolphthalein as an indicator to the filtrate and FFA value was calculated as follows:

$$\text{FFA (\%)} = \frac{(\text{mL titration} \times \text{Normality of KOH} \times 28.2)}{\text{g of sample}}$$

Protein oxidation

Tyrosine value

First, a trichloro acetic acid extract for measuring tyrosine value was made by blending 20 g of meat for 2 minutes with 50 ml of cold 20% trichloro acetic acid. The blended contents are rinsed with 50 ml of distilled water and filtered through Whatman No. 1 filter paper. The filtrate volume is then collected in a measuring cylinder with a 100 ml capacity. In a test tube, 2.5 ml of TCA extract was diluted with an equal volume of distilled water. Then, 10 ml of 0.5 N sodium hydroxide and 3 ml of diluted folin ciocalteu phenol reagent (one-part folin ciocalteu phenol reagent to two parts distilled water) were

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added to the mixture. After mixing, it was left at room temperature for 15 minutes. Using a blank (5 ml of 5% TCA) as a reference, the developed blue color was measured as an absorbance value at 660 nm in a spectrophotometer. Tyrosine content was determined and expressed as mg of tyrosine/100 g of meat sample using the standard graph.

Sulphydryl groups (thiol content)

For determining SH group level, 0.5 ml of meat homegenate was mixed with 2.5 mL of Tris-Gly-8M Urea and 0.02 mL of 4 mg/mL 5, 5- dithiobis-2,2-nitrobenzoic acid (DTNB). After incubation at 25 °C for 30 min, the absorbance at 412 nm (A412) was recorded. The SH group level was calculated by equation, SH group level (μmol/ g proteins).

$$A = \frac{D}{C}$$

Where;

A is Sample reading at 412 wave length

D is the dilution coefficient (6.04)

C (mg/mL) is the protein concentration in tested sample.

(6.04); C (mg/mL) is the protein concentration in tested sample.

Shelf life

Total viable count

Using a sterile pipette with plastic tips, 1gm of pre-prepared samples from dilutions of 10^{-3}, 10^{-4}, and 10^{-5} were transferred into sterile petri dishes. Subsequently, 15ml of sterile plate count agar medium was added to each dish. Rotating the petri dishes in both counterclockwise and clockwise directions facilitated thorough mixing of the inoculum. After setting up, the dishes were inverted and incubated at 30°C for 72 hours. Colonies on selected dishes, ranging between 30 and 300, were then counted using a colony counter by using specific formula;

$$N = \frac{\sum \mathbf{C}}{(\mathrm{n1} + 0.1\,\mathrm{n2})\mathrm{d}}$$

Where;

∑C= sum of colonies counted on all the dishes retained.

n1= no. of dishes retained in first dilution.

n2= no. of dishes retained in second dilution.

d= dilution factor corresponding to first dilution.

Total coliform count

The Coliform count was determined following the method outlined by the British Standard Institution (BSI, 1993). After transferring pre-prepared meat samples (1gm) from dilutions of 10^{-2}, 10^{-3}, and 10^{-4} into sterile petri dishes using a dispensing pipette with sterile plastic tips, 12ml of sterile violet red bile agar medium was introduced. The mixture was thoroughly blended, allowed to solidify, and then incubated at 37 °C for 24 hours. Subsequently, selected dishes containing colonies ranging from more than 10 to fewer than 200 were counted using a colony counter. The results were translated using the same formula (as for Total Viable Count).

Statistical analysis

The study use statistical analysis, utilizing ANOVA to assess means across multiple groups. Subsequently, a post-hoc LSD test was employed to pinpoint significant

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differences between pairs of individual groups.

Results and Discussion

Thiobarbituric acid (mg MDA/kg**)**

The thiobarbituric acid was observed and result are shown as below in figure 1. The thiobarbituric acid in control group was 0.11, 0.13, 0.15, and 0.18, respectively at 00, 24, 48 and 72 hour interval. Whereas, in yoghurt, ginger extract, tamarind juice, honey 20%, 25%, and 30% at 24 hour marination was 0.13, 0.14, 0.16, 0.12, 0.15, and 0.16, respectively and for 48 hour, 0.14, 0.15, 0.17, 0.13, 0.16, and 0.17, respectively while at 72 hour was 0.15, 0.16, 0.18, 0.15, 0.18, and 0.18 respectively. The study observed varying levels of thiobarbituric acid in different groups over 72 hours. The marinades, specifically yoghurt, ginger extract, tamarind juice, honey 20%, 25%, and 30%, showed different trends at 24, 48, and 72 hours. Specifically, at 72 hours, the control group displayed a significant difference (P≤0.05) compared to fresh meat values. Moreover, fresh meat results demonstrated high significance with tamarind juice and honey 30% at various storage intervals under refrigeration conditions. Overall, as the storage period increased, both the control and marinades exhibited elevated levels of thiobarbituric acids in the study.

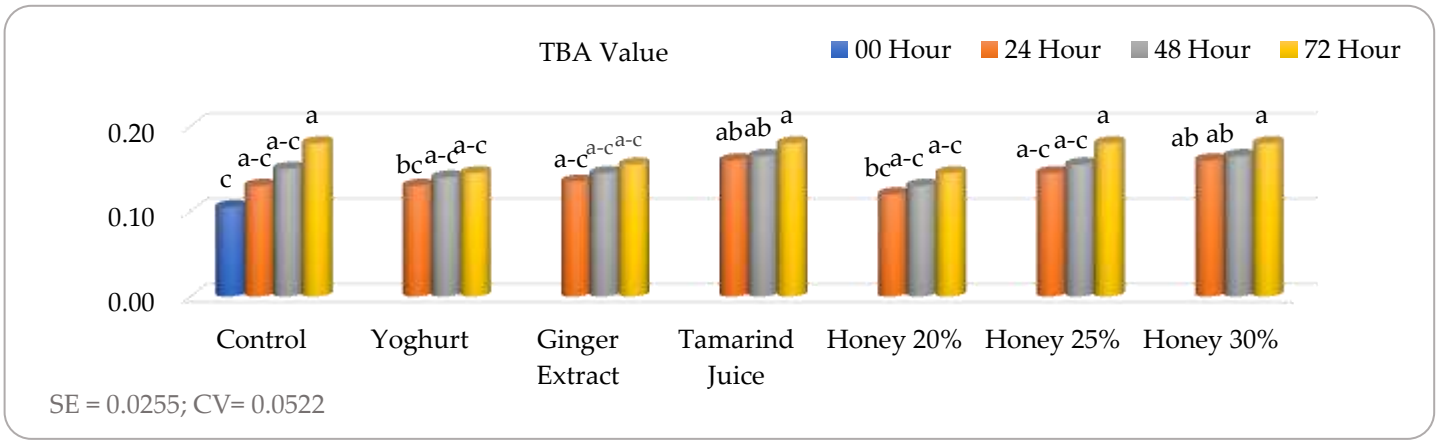

Figure 1. Comparison of the thiobarbituric acid content values by using different marinades of spent hen meat with time interval.

Free fatty acids (%)

In various marinades, free fatty acids was observed, and results showed increased trend in various treatments (Figure. 2). These results were 0.95, 1.00, 1.12, and 1.15, respectively in control group at interval i.e. 00, 24, 48 and 72 hour. At 24 hour of duration, results in yoghurt, ginger extract, tamarind juice, honey 20%, 25%, and 30% were 0.88, 0.81, 0.85, 0.78, 0.78 and 0.94, respectively. While at 48 hour the findings were 0.92, 0.88, 0.95, 0.85, 0.85, and 0.96, respectively. At storage period of 72 hour, it was 0.95, 0.93, 0.98, 0.88, 0.93, and 0.99, respectively. The analysis of free fatty acids in various marinades revealed an increasing trend across different treatments. Notably, fresh meat values exhibited a non-significant (P≥0.05) difference with all marinades. The lowest free fatty acids value was observed in honey 20% marination, followed by ginger extract, yoghurt, tamarind juice,

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honey 25%, and honey 30%, respectively, in comparison to the control group.

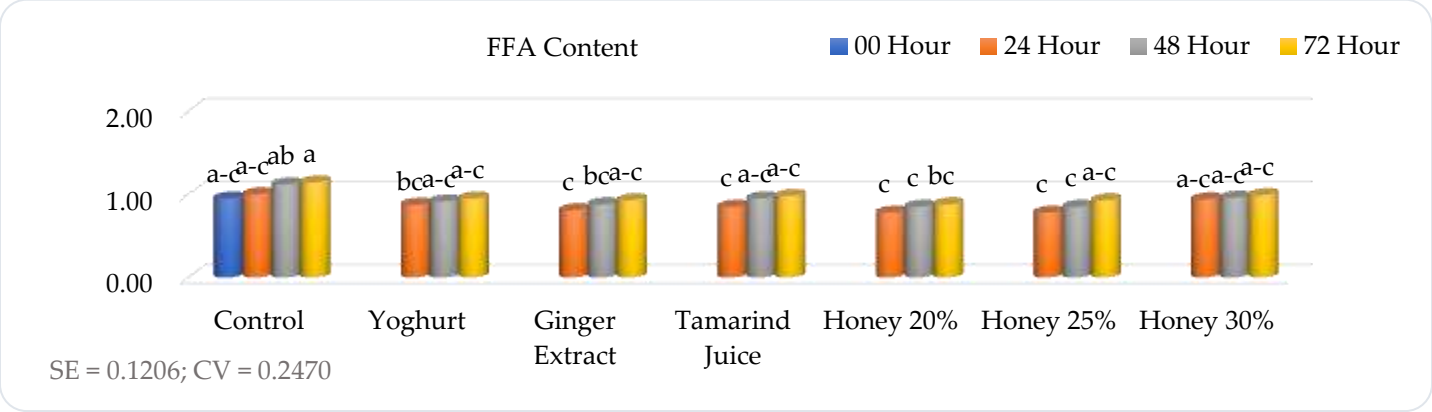

Figure 2. Comparison of the free fatty acid content values by using different marinades of spent hen meat with time interval.

Peroxide value (meq/kg)

The result of peroxide value (POV) is shown in figure 3. In the control group, POV at 24, 48, and 72 hours of refrigerated storage were 1.67, 1.75, 2.05, and 2.14, respectively. For various marinades (yoghurt, ginger extract, tamarind juice, honey 20%, 25%, and 30%) after 24 hours of marination, the POV values were 1.57, 1.62, 1.68, 1.62, 1.88, and 1.98. At 48 hours, results were 1.70, 1.79, 1.80, 1.77, 1.95, and 2.05, and at 72 hours, they were 1.90, 1.95, 1.88, 1.83, 2.06, and 2.10, respectively (Figure 3). The fresh meat values exhibited non-significant (P≥0.05) differences with all marinades at different intervals, except 72 hour control and honey 30% respectively. Overall, there was an increase in peroxide values across all treated groups.

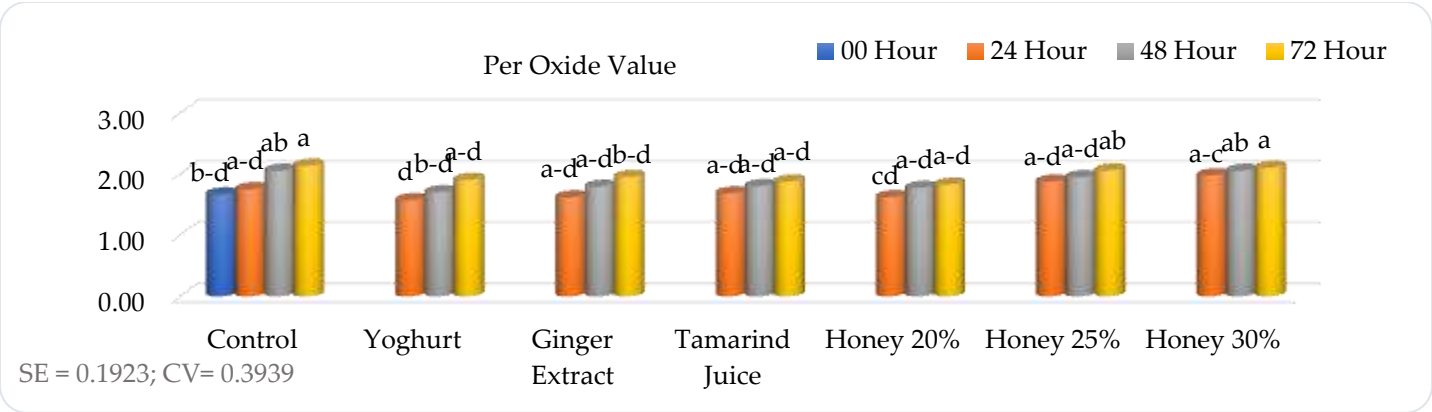

Figure 3. Comparison of the peroxide value content values by using different marinades of spent hen meat with time interval.

Tyrosine value (tyrosine/100g)

In fresh spent hen meat, the tyrosine value was 0.65 at the 00 storage time (Figure 4). Subsequently, at 24, 48, and 72 hours of storage, the values rose to 0.80, 0.93, and 1.13, respectively. Furthermore, in marinades such as yoghurt, ginger extract, tamarind juice, honey 20%, 25%, and 30% during the 24 hour marination period, the tyrosine values were 0.65, 0.69, 0.78, 0.64, 0.83, and 0.85, respectively. At 48 hours, these values increased

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to 0.76, 0.84, 0.93, 0.72, 1.01, and 1.05, and at 72 hours, they were 0.83, 0.95, 1.05, 0.83, 1.07, and 1.13, respectively. The tyrosine content in fresh meat exhibited a non-significant (P≥0.05) difference with all marinades at the 24 hour interval. However, at the 48 hour storage period, significant (P≤0.05) differences were observed with the control, tamarind juice, honey at 25%, and honey at 30%, respectively. The 72-hour storage period showed significant (P≤0.05) differences with the control, ginger extract, tamarind juice, honey 25% and honey 30%, respectively except yoghurt and honey 20%. These results indicate an increase in tyrosine content in both the control and marinaded groups.

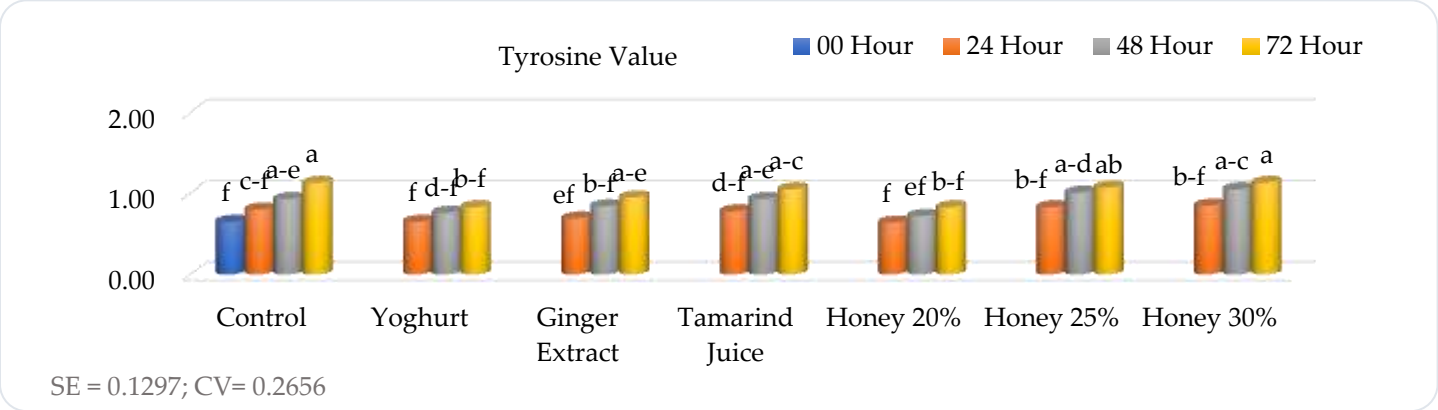

Figure 4. Comparison of the tyrosine value by using different marinades of spent hen meat with time interval.

Sulfhydryl value (µmol/g protein)

The impact of various marinades on sulfhydryl content was examined and shown in figure 5. Initially, the fresh meat value was 2.17 at 00 hours of storage. Subsequently, at the 24 hour, the values for the control, yoghurt, ginger extract, tamarind juice, honey 20%, 25%, and 30% were 2.10, 2.05, 2.12, 2.10, 2.17, 2.08, and 2.05, respectively, whereas at 48 hour interval, these values changed to 1.93, 1.95, 1.98, 2.09, 1.99, and 1.98 respectively. After 72 hours of refrigeration, the findings were 1.85, 1.90, 1.87, 1.90, 1.95, 1.85, and 1.85 respectively (Figure 5). The fresh meat value showed non-significant (P≥0.05) difference with the 24 hour control group and all marinade groups. However, a significant (P≤0.05) difference was observed in all marinade groups at the 48 and 72 hour storage, except honey 20% at the 48 hour storage. A decreasing trend in sulfhydryl content was observed in both the control and all other groups.

Total viable count (log cfu/g meat)

The impact of different marinades on the shelf life of meat was observed and is illustrated in figure 6. In the control group, initial findings at 00 hours were 6.12, and at subsequent 24, 48, and 72-hour intervals, values increased to 7.19, 8.37, and 10.31, respectively. For yoghurt, ginger extract, tamarind juice, honey at 20%, 25%, and 30%, the values at 24 hours were 5.28, 5.81, 6.92, 5.45, 6.23, and 7.08, respectively. Results at 48 hours were 6.05, 6.03, 7.93, 5.80, 7.05, and 7.90, and at 72 hours, these were 7.50, 7.11, 8.35, 6.75, 7.50, and 9.00, respectively. Significant (P≤0.05) differences were observed in fresh meat values with the various marinades, and significant (P≤0.05) differences were also noted in the control group at different intervals. The total viable count increased in the

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control group, while a less pronounced increase was observed in the treated group.

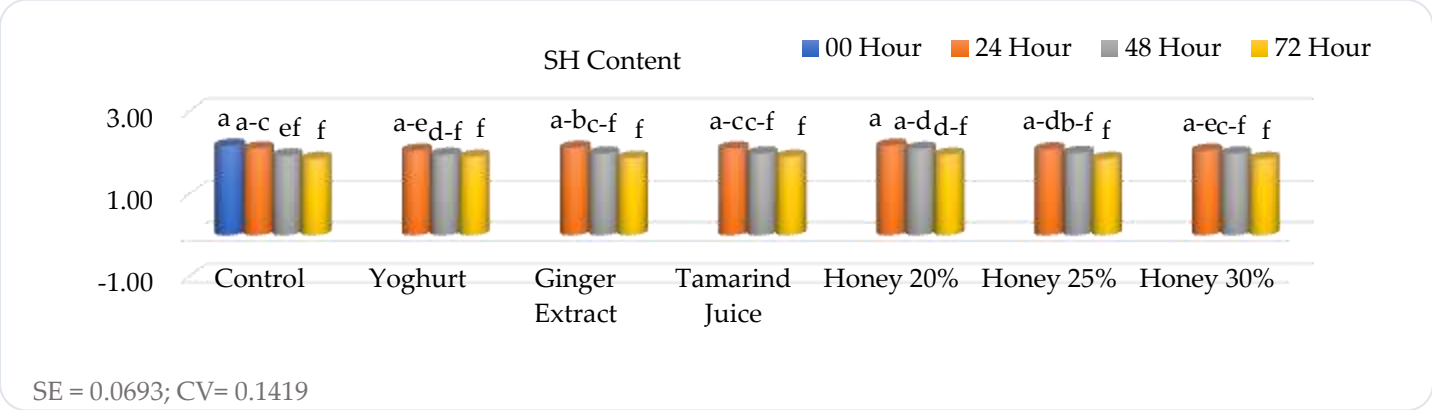

Figure 5. Comparison of the sulfhydryl value by using different marinades of spent hen meat with time interval.

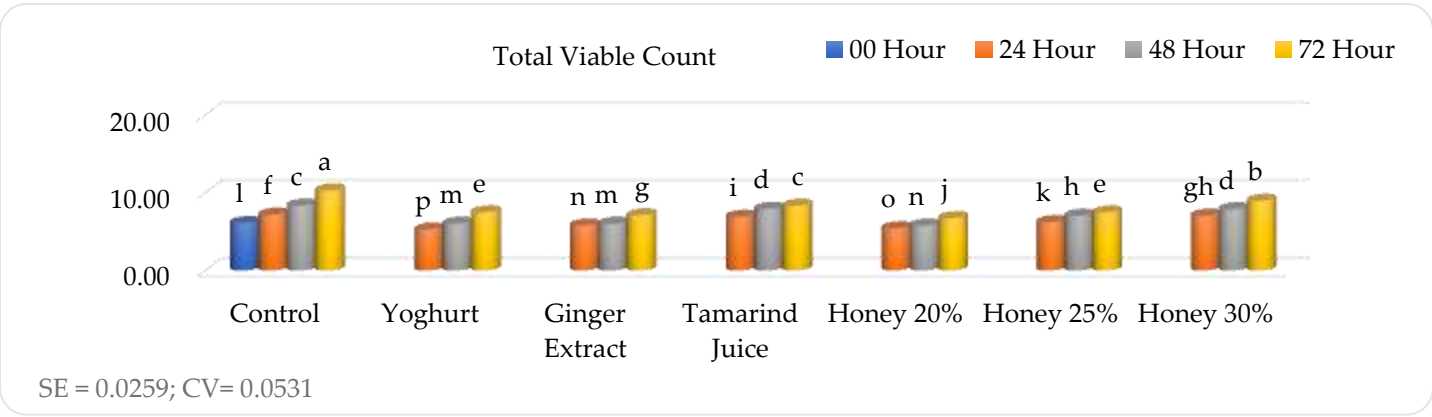

Figure 6. Comparison of the total viable count by using different marinades of spent hen meat with time interval.

Coliform count (log cfu/g meat)

The effect of different marinades on shelf life of spent hen meat was observed as shown in figure 7. Coliform count in fresh meat was 4.71 at 00 hours of storage, while at 24 hour, these were 5.66, 5.88, 4.08, 5.22, 3.50, 5.10, and 5.76, respectively, at 48 hour of storage time, coliform count was 6.50, 6.25, 5.50, 5.51, 5.63, 6.17, and 6.44, respectively, and at 72 hour of storage interval, the results were 6.70, 6.35, 6.71, 6.55, 5.75, 6.50, and 6.48, respectively, in control, yoghurt, ginger extract, tamarind juice, honey 20%, 25% and 30%, respectively (Figure 7). The fresh value was significant ($P \leq 0.05$) statistically. Specifically, an increasing trend in coliform counts was observed in the control group compared to the treated groups, indicating a potential positive effect of the marinades in extending the shelf life of spent hen meat.

Discussion

The increase in TBA (Thiobarbuturic acids) levels in the control group indicates a progression of lipid oxidation over the storage period, leading to potential deterioration in meat quality. The variations in TBA levels among marinades at 24 hours suggest differences in their ability to inhibit lipid oxidation, with some showing potential

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protective effects. The significant difference observed with tamarind juice and honey 30% in fresh meat values at various intervals indicates these marinades might have antioxidative properties. The increase in TBA levels in control and marinaded groups over time highlights the susceptibility of spent hen meat to lipid oxidation during refrigerated storage. The results recommend that the storage period increases the thiobarbituric acid levels in both control and marinaded spent hen meat. Different marinades may have varying effects on inhibiting lipid oxidation, and further research is required to elucidate the specific antioxidative properties of each marinade.

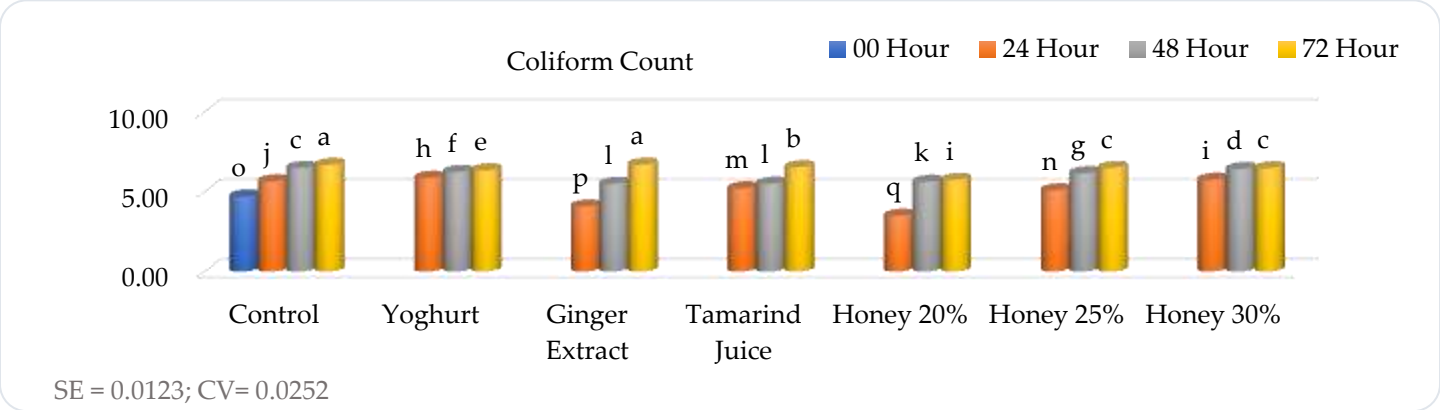

Figure 7. Comparison of the coliform count by using different marinades of spent hen meat with time interval.

At the end of the time interval, the TBA (mgMDA/Kg) values of all marinades were significantly lower than those of the control group. The control group showed a range of 0.11 to 0.18 mg MDA/kg during storage at 0 to 72 hours of refrigeration. Whereas, the yoghurt marination was 0.13 to 0.15, ginger extract was 0.14 to 0.16, tamarind juice was 0.16 to 0.18, honey 20% was 0.12 to 0.15, honey 25% was 0.15 to 0.18, and honey 30% was 0.16 to 0.18. Among the marinades, the honey 20% marinade exhibited the lowest TBA value, suggesting it had the least lipid oxidation compared to both the control and treated groups. This aligns with findings from previous studies by Gupta and Sharma (2016) and Biswas *et al.* (2006), where increased TBA levels were observed in stored meat particularly in the interval of 00 to 03 days. The consistency in results were also noted with observation of Yadav *et al.* (2018).

The increase in free fatty acids (FFA) in both control and marinated groups over time suggests the occurrence of lipolysis, leading to the release of free fatty acids. The variations in free fatty acid levels among different marinades at 24, 48, and 72 hours may be attributed to the different compositions and effects of each marinade on lipid degradation. The non-significant differences in fresh meat values suggest that the initial marination process did not induce significant changes in free fatty acids compared to untreated meat. According to Rokib *et al.* (2019), storage time has a significant impact on free fatty acids (FFA). High FFA is somewhat associated with unacceptability, even though FFA formation does not necessarily signify nutritional loss. According to Miranda *et al.* (2016), FFA accelerates the development of off-flavor and has a negative impact on muscle texture. The initial free fatty acid (FFA) content in fresh samples was

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0.55, but a rising trend was observed in both the control and marinated samples after 24, 48, and 72 hours of marination. Nazeri *et al.* (2018) found a similar increasing trend in FFA values with a 0.3% marination of pistachio green hull extract, comparable to the trends in the control and other marinades.

The control group showed a significant increase in peroxide values over the storage period, indicating potential rancidity. Marinades, including yoghurt, ginger extract, tamarind juice, and honey at different concentrations, did not show significant differences in peroxide values compared to fresh meat, except at 72 hours for the control group and honey 30% concentration. The overall increase in peroxide values in treated groups advises that the marinades did not prevent the development of rancidity and may have contributed to its progression over time. This value agreed in respective time intervals of 24, 48, 72 hours with Ayoob *et al.* (2023) and Hossain *et al.* (2021) results in respect of time and concentration of marinades. The Per oxide value (meq / kg) was observed in fresh spent hen meat 1.67 closely associated with the result of Goswami *et al.* (2014) who's finding in minced chicken meat was 1.366, while 2.450 (3rd Day) of storage at 4Ċ which was agreed with trend but dis agreed with value, might be because of minced meat and raw meat marination and specie, age difference as in our results with time interval the increased value observed in control and marinades. In the study, Kaleri *et al.* (2023) noted an increasing trend in marinades, with pistachio green hull extract (PHE) at 0.3% aligning significantly with the control group. Dzudie *et al.* (2004) found that ginger's antioxidant properties matched the results of ginger extract. Cheok and Chin (2012) explored the impact of salt and tamarind juice on beef satay, aligning with the findings related to tamarind juice.

The increase in tyrosine content over the storage period in both fresh meat and groups indicates a potential breakdown of proteins, possibly due to microbial or enzymatic activity. The differences observed at 48 and 72 hours may indicate that certain marinades influence the rate of protein breakdown in spent hen meat during storage. The non-significant differences at 24 hours suggest that, initially, the marinades may not have a significant impact on tyrosine content compared to fresh meat, the findings recommend a time-dependent increase in tyrosine content in both fresh and marinaded groups, with variations influenced by different marinades and concentrations. The study observed a tyrosine content of 0.65 in fresh spent hen meat, with an increasing trend in the control group over time. The results from yoghurt, ginger extract, tamarind juice, and honey concentrations (20%, 25%, 30%) aligned with Chuaqui-Offermanns and McDougall (1991). Thanatsang *et al.* (2020) reported different tyrosine content in broiler breast, potentially influenced by breed or age. Anandh (2014) noted elevated tyrosine content in refrigerated chicken meat, correlating with varied storage intervals. Triki *et al.* (2018) and Lee *et al.* (2012) found higher tyrosine content in chicken meat, aligning with the results from both control and all marinaded groups, indicating a significant relationship.

The decrease in sulfhydryl content in both the control and marinade groups over time proposes a potential degradation of protein structures. The non-significant differences at 24 hours indicate that initially, marinades did not have a significant impact on sulfhydryl content compared to fresh meat. The significant differences observed at 48 and 72 hours

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suggest that the marinades may influence the rate of sulfhydryl content degradation during prolonged storage. The decrease in sulfhydryl content may be indicative of protein denaturation or other chemical changes occurring during storage and marination. The significance of the differences between control and marinade groups at 48 and 72 hours warrants attention and could be related to the composition of the marinades. The sulfhydryl value (SH) significantly decreased in both the control and various marinades, but the decline was less pronounced in the marinades. Every marinade, including the control, exhibited a declining trend in SH content. Results were consistent with Vaithiyanathan *et al.* (2011), reporting total-SH content in spent hen breast meat, and with Hofmann and Hamm (1978), who observed similar trends in chicken and turkey breast meat.

The significant increase in total viable count (TVC) in the control group indicates a progressive increase in microbial contamination and spoilage over the storage period that influences the quality of meat for consumption. The treated groups (marinades) appear to have a preservative effect, as evidenced by lower TVC values at 24, 48, and 72 hours compared to the control group. The lower TVC values in the treated groups indicate a potential antimicrobial effect of the marinades, contributing to an extended shelf life of the meat. The significant differences between fresh meat and marinade groups suggest that the marination process has a positive effect on inhibiting microbial growth. The control results matched with Aydin *et al.* (2007), indicated TVC values in ground meat with garlic addition, similar trends was observed in the control and various marinades, including yoghurt, ginger extract, tamarind juice, and 20% honey at 24 and 48 hours. TVC values of 3% ginger extract in chevon meat (Pawar *et al.*, 2007) and garlic juice on beef (Nurwantoro *et al.*, 2011) were in agreement with the study's findings. Ayoob *et al.* (2023) reported TVC in honey-marinated beef, correlating with the current study. Honey's antibacterial properties, influenced by hydrogen peroxide production, as noted by Jenkins *et al.* (2014) results were corelates with the observed effects in honey marinades. Overall, the study suggests that various marinades, especially those with honey, ginger extract, and tamarind juice, can influence microbial activity in spent hen meat.

The statistically significant difference between groups indicates that the marinades have a positive effect in reducing coliform counts, contributing to a potential extension of the shelf life of spent hen meat. The increased trend in the control group indicates a higher level of microbial contamination and suggests a potential role of the marinades in controlling microbial growth. Variations in coliform counts among different marinades may be attributed to the specific antimicrobial properties of each marinade and observed reduction in treated groups proposes a potential antimicrobial effect of the marinades, contributing to improved meat safety and shelf life. The coliform count increased in the control group, while in marinades, the increase was comparatively less pronounced over time. The study conducted by Dogan *et al.* (2014) examine the coliform count (3.00 log cfu/g) closely resembling the count associated with 20% honey at the 24 hours. Aydin *et al.* (2007) observation (5.77 log10 cfu/g) correlated with the control group, yoghurt, tamarind juice, and honey 25% and 30%. Ayoob *et al.* (2023) reported coliform results in

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honey-marinaded beef that aligned with the results obtained with 20% and 25% honey. The increasing trend observed in the control group and marinades correlated with the findings reported by Das *et al.* (2022).

Conclusion

In the context of lipid oxidation, the thiobarbituric acid, free fatty acids, and peroxide values showed an increasing trend with the storage period. Conversely, in protein oxidation increased tyrosine and decreased sulfhydryl content was observed across all groups. In assessing the shelf life of spent hen meat, the total viable count and coliform count exhibited an increased trend. Despite these observed trends, the overall impact of marination was positive, as it effectively alleviated oxidation and prolonged the shelf life of spent hen meat, when compared to the control group. Further investigations could explore the specific factors influencing these trends, such as environmental conditions, storage parameters, or the impact of any applied treatments. This information would contribute to a more comprehensive understanding the processes and provide valuable vision for potential strategies in quality preservation of meat.

Conflict of Interest

There is not any conflict of interest to authors.

Authors Contributions

All the authors equally contributed to manuscript.

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