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Effect of cold storage on lipid and protein oxidation, microbial spoilage, and physicochemical properties of chicken meat.

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ABSTRACT

Due to the low-fat content and its richness in fatty acids, especially the saturated ones, and lower cholesterol levels than mutton and beef, many consumers prefer broiler chicken meat. However, the refrigerated storage may lead to chemical alterations and microbial proliferation in the meat product, generating metabolites that induce physical and chemical quality changes in the chicken meat. Thus, the study focuses on chicken meat's oxidation of lipids and proteins, microbial degradation, and physicochemical attributes during refrigeration. The study involves 30 forty-two-day-old ROSS broiler chickens with an average live body weight of 2.023 ± 0.017 kg at a commercial plant in Erbil, Kurdistan region of northern Iraq. After bleeding and evisceration, chicken breast muscle samples were divided into three portions, then tagged, vacuum-sealed, and stored in a 4°C refrigerator for 1, 3, and 5 days. In this study, there were increased activities of microbes, including lactic acid bacteria, Enterobacteriaceae, pseudomonas species, and total aerobic counts ($P \leq 0.05$) as the days of ageing went up. The thiobarbituric acid reactive substances, carbonyl, and free thiol contents changed significantly as the meat ages for 1, 3, and 5 days. Similarly, the refrigerated storage of broiler chicken meat was significantly reduced the value of colour, muscle pH, WHC, drip loss, cooking loss, and heavy chains (actin and myosin). Improved refrigerated storage and meat ageing are vital to ensuring better meat quality, safety, and shelf life.

***Keywords*:** oxidation, microbial deterioration, meat quality, chicken meat, and refrigeration storage.

INTRODUCTION

The global poultry industry is expanding and evolving to satisfy consumer expectations. The quality of poultry meat. A report by FAO [1] suggests that poultry production has risen from 56 million to 119 million, representing an increase of over 100 per cent in the last two and a quarter decades. On the other hand, the postmortem factors pertain to the refrigerated storage of meat, commonly known as maturation or ageing [2]. Due to the low-fat content and its richness in fatty acids, especially the saturated ones, and lower cholesterol levels than mutton and beef, many consumers prefer broiler chicken meat. Despite its low lipid content, chicken meat contains a considerable amount of unsaturated fatty acids and serves as a source of chain-elongated linoleic acid.

The change of muscle to meat and quantitative alterations involving a cascade of events via metabolism occur during the ageing process. Consequently, if spoilt, meat is unfit for human ingestion [3]. Individual sensory acuity, the intensity of the change, and cultural and economic factors typically influence spoilage [4]. Suggest that bacteria levels between 6 and 7 log CFU/g significantly influence meat deterioration during refrigerated storage [5]. According to [6], thiobarbituric acid reactive substances (TBARS) at 5 mg malondialdehyde/kg of meat are the level that shows the meat is safe for humans to eat. [7] state that refrigeration and thawing severely impact lipid and protein oxidation, which entails several biological modifications influencing meat quality, including colour and pH [8]. Furthermore, the chemical alterations have an adverse impact on meat colouration and tenderness while also decreasing water-holding ability. Ensuring the microbiological quality and safety of poultry meat continues to be a global challenge for the meat industry. This is because of several natural and processing factors, such as its high pH (> 6.0) and the fact that the digestive tract is close to the muscle tissue in birds, which makes it more likely that spoilage or pathogenic enteric bacteria will get into the meat during slaughter and evisceration. Post-slaughter refrigeration of chicken is crucial to inhibit microbial proliferation and prevent lipid-protein oxidation deterioration [9]. Nonetheless, prolonged refrigerated storage may lead to chemical alterations and microbial proliferation in the meat product, generating metabolites that induce physical and chemical quality changes in the chicken meat [10].

Current research acknowledges the essential influence of storage temperature on preserving optimal meat quality and safety before consumer distribution. In general, freezing does not reduce the microbial population in meat. Rottenness remains suspended owing to the inactivity of the microorganisms. An amalgamation of effective hygiene and handling protocols and appropriate temperature regulation will reduce pathogenic microbial populations [10].

This study aims to determine the changes in the physicochemical properties and quality of chicken breast meat following storage at a chilling temperature of 4°C. These include lipid-protein oxidation, microbiological quality, and technological characteristics.

Material and methods

Carcass sampling and storage

In this study, 30 forty-two-day-old male Ross broiler chickens reared under a similar management system, with an average live body weight of 2.023 ± 0.017 kg, were purchased from a commercial poultry farm. We slaughtered the birds per halal slaughter procedures. The commercial poultry abattoir (Al Eatemad slaughterhouse, Kurdistan Region of Iraq) conducted the slaughter procedure. A licensed slaughterman conducted the halal slaughter. A licensed slaughterman pulled each bird's head dorsally to stretch the neck and facilitate exsanguination. We used a sharp knife to cut a transverse section. The neck incisions penetrated the outer layer of skin, muscle, oesophagus, trachea, carotid arteries, jugular veins, and significant nerves without resulting in decapitation.

We extracted and segmented chicken breast muscle samples into three portions: 1, 3, and 5 days after bleeding and evisceration. We tagged, vacuum-sealed, and stored them in a 4°C refrigeration unit. After a designated five-day storage period, we extracted all Pectoralis major muscle samples from their packages and sectioned them into various portions for microbiological analysis and meat quality assessments. We rapidly froze the lipid-protein and myofibrillar protein study samples in liquid nitrogen, pulverised, vacuum-sealed, and stored at -20°C until analysis.

Microbiological Analysis: We carefully weighed 1 gramme of Pectoralis major muscle samples on days 1, 3, and 5, placed them in a plastic centrifuge tube with 10 ml of deionised water, and mixed them at room temperature for 120 seconds. For microbial enumeration, we spread 100 µl samples of a 10-fold dilution in deionised water on the surface of dry media. We used the tenfold dilution on two sets of Petri dishes to count the total aerobic count (TAC) on Neogen-NCM0033A plate count agar. We incubated the plates at 32°C for 24 hours. After incubation, we counted all bacterial colonies (cfu/g) on TCB plates and converted them to log10 CFU/g for statistical analysis [10].

Assessment of Thiobarbituric Reactive Substances: After weighing and combining the samples in a buffer solution like trichloroacetic acid (TCA), we manually pulverized the breast muscle samples using a mortar and pestle. The meat sample is homogenised with an appropriate solvent, often an aqueous solution of TCA (e.g., 10-15%), to precipitate proteins and release lipid oxidation products. After homogenisation, the sample undergoes centrifugation to segregate the solid meat particles from the liquid that contains the extracted lipids and oxidation byproducts. The supernatant is collected, and an equivalent volume of thiobarbituric acid reagent is included. TBA generally interacts with malondialdehyde (MDA), a key marker of lipid peroxidation. The sample extract and TBA reagent mixture are heated at 90°C for 60 minutes, creating a pink-hued complex between MDA and TBA. After incubation, the sample is allowed to cool to ambient temperature. The intensity of the pink hue, which coincides with the concentration of MDA, is quantified using a spectrophotometer (Spectronic Instruments, USA). Absorbance is typically assessed at 532 nm. The concentration of MDA is determined by comparing the absorbance to a standard calibration curve of known MDA concentrations. MDA is frequently quantified as milligrammes of MDA per kilogramme of meat sample (mg MDA/kg) as per the description of Abubakar et al. [11].

Assessment of Protein Oxidation

Meat protein oxidation was evaluated by measuring the amount of thiols (the sulfhydryl group (SH) of a cysteine residue) (Winterbourn, 1990) with slight modification [12]. We quantified the free thiols and carbonyl group contents using a colorimetric assay kit (ABCAM, USA) according to the manufacturer's instructions. We calculated and quantified the results in nanomoles per milligrammes of free thiols and protein carbonyl concentrations.

Immunoblotting of Myofibrillar Proteins

We used immunoblotting to get the myofibrillar proteins out of the cells, following the steps outlined by [13] and slightly changed by [12]. We mixed about 2.5 g of samples from the pectoralis major in 20 ml of extraction buffer containing 150 mM NaCl, 25 mM KCl, 3 mM MgCl2, and 4 mM Ethylenediaminetetraacetic acid (EDTA). We set the pH at 6.5 for 30 s and added protease inhibitors (Sigma Aldrich, Germany) to the homogenates. We filtered the homogenates to remove the leftover collagen. Following the filtering process, we incubated the homogenate at 4oC, shook it, and centrifuged it at 2000 g for 15 minutes at 4oC. We washed the supernatants twice: once with 25 ml of 50 mM KCl solution at a pH of 6.4 and once with 25 ml of mM phosphate buffer at a pH of 6. We resuspended the supernatants in phosphate buffer and stored them at -20°C until our subsequent analysis.

Extraction of Myofibrillar Proteins

We measured the protein contents in the suspension using a test kit (Parsazmoon, Iran) and the colourimetric analytical techniques of Bradford [14]. We used bovine serum albumin (BSA) to create a protein measuring standard. For standard 0.0, we diluted the BSA into six (6) dissimilar concentrations. 0.1, 0.3, 0.4, and 0.5 µg/µl with the same buffer used to extract proteins. Ten microlitres of the sample and the standard were put into each well of the 96-well plate in duplicate. Then, 200 microlitres of Bradford dye reagent were added, and the plate was left to sit at room temperature for five minutes. A microplate reader (Awareness Stat Fax 2100, USA) detected the absorbance at a wavelength of 595 nm. We plotted a standard curve and used its derived equation to determine the protein concentration [10].

Sodium Dodecyl Sulfate-Polyacralamyde Gel Electrophoresis (SDS-PAGE)

A technique widely used to separate proteins based on their molecular weights. The technique relies on the ability of sodium dodecyl (SDS), an anionic detergent used for denaturing proteins by imparting a negative charge that is proportional to their length.

Denaturing proteins

Proteins are combined with SDS and a reducing agent, such as β-mercaptoethanol or dithiothreitol, in a buffer solution. The SDS denatures proteins by destroying their tertiary and secondary structures, rendering them linear. The reducing agent cleaves disulphide bonds in the protein, guaranteeing total denaturation. Heating: We generally heat the protein-SDS combination to around 95°C for 3-5 minutes to guarantee thorough denaturation and interaction between SDS and the protein. This phase facilitates the consistent unfolding and coating of proteins by SDS molecules. Preparation of Gel: Polyacrylamide Gel Fabrication: We synthesise the gel from polyacrylamide, which creates a permeable matrix. Ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) facilitate the polymerisation of acrylamide and bis-acrylamide. The concentration of acrylamide can be modified to produce gels with different pore sizes, influencing protein separation resolution. SDS-PAGE gels consist of two components: stacking and resolving gels. Stacking Gel (upper section, pH ~6.8): A minimal acrylamide concentration concentrates the proteins into a narrow band before entering the resolving gel. Resolving Gel (lower section, pH ~8.8): This gel contains a greater concentration of acrylamide and functions to separate proteins according to size. Sample Ingestion: Wells inside the Gel: The stacking gel creates wells for introducing protein samples upon polymerization. Each well can hold a limited volume of the sample. Molecular Weight Markers: We introduce molecular weight standards or protein ladders in a distinct well adjacent to the samples. These comprise proteins of established molecular weight, functioning as a benchmark for assessing the size of the proteins under examination. Electrophoresis: Buffer System: The gel is immersed in an electrophoresis running solution, often Tris-Glycine-SDS buffer, to supply ions for current conduction and to stabilise pH levels. The SDS in the solution guarantees that proteins remain denatured and consistently charged [10].

Application of Electric Field

A steady voltage, typically ranging from 100 to 200 V, is applied across the gel, resulting in the migration of negatively charged proteins towards the anode (positive electrode). Protein Migration: Due to SDS imparting a uniform charge-to-mass ratio to all proteins, their migration through the gel is predominantly influenced by their size. Smaller proteins migrate more rapidly through the polyacrylamide matrix, whereas larger proteins exhibit slower movement due to increased resistance [10].

Gel Staining

Protein Visualisation: Upon concluding electrophoresis, we apply a dye to the gel to visualize the separated proteins. Coomassie Brilliant Blue is the predominant stain, binding to proteins and producing discernible bands. Alternatively, one may employ more sensitive stains such as silver staining or fluorescent dyes to identify minimal quantities of protein [10].

Destaining

The gel is washed in a destaining solution, often a combination of methanol and acetic acid in water, to eliminate surplus stain that fails to adhere to proteins. This makes the protein bands distinctly visible against the gel's transparent background. We examine the gel to ascertain the location and intensity of the protein bands. We ascertain the molecular weight of the proteins by relating their migration distance with that of the molecular weight markers. We create a graph of the logarithm of molecular weight (known for the markers) against migration distance to estimate protein size [10].

Muscle pH Determination

Before analysing the samples, calibrate the ph meter at 4.0 (acid) and 7.0 (neutral). We assessed the samples from the pectoralis major of broiler chickens using an indirect method and a handy pH meter (EZDO PP-203, Taiwan). We added approximately 1 g of the manually crushed samples and 20 ml of ice-cold deionised water, homogenised them for 30 seconds, and then read the samples by immersing the product inside the beaker containing the homogenates [15].

Water Holding Capacity

We determined the pectoralis major's water-holding capacity (WHC) for cooking and drip loss using techniques described by [15]. We collected approximately 20 g of fresh samples, weighed them, and noted X1 as the initial weight for the drip loss. We kept the samples in polyethene bags, labelled them, vacuum-packed them, and placed them in a 4 °c chiller for five days. After completing the postmortem ageing days, we removed the samples from the polyethylene bags, gently wrapped them in tissue paper to eliminate excess moisture, weighed them, and recorded the results as X2. The amount of drip loss was calculated using the formula below: drip loss (%) = [(X1-X2) ÷ X1] × 100. For cooking loss assessment, samples obtained earlier from the pectoralis major of broiler chickens were weighed (X1), and values were noted and vacuum-parked. We filled a water bath (HAAKE C10, UK) with water, preheated it to 80oC, and then placed the samples in it for 10 minutes, monitoring the internal temperature with a temperature probe until it reached 78oC. After 10 minutes, we removed the samples and cooled them over tap water until they reached room temperature. After removing the samples from the polythene bags, we gently wrapped them in tissue paper to absorb the excess water. Once dry, we reweighed the samples and recorded them as (X2). As shown below, the cooking loss was found by multiplying (X1-X2) by X1 and multiplying that number by 100.

Warner-Bratzler Shear Force Evaluation of Broiler Chicken Meat

A Volodkovitch bite jaw connected to a Brookfield Texture Analyser (CT3TM, USA) was used to measure the Warner-Bratzler shear force (WBSF) of the pectoralis major muscle of broiler chickens. We calibrated the texture analyser at a speed of 10 mm/s and a return distance for a height of 10 mm. We prepared the samples according to [16] description. We prepared the samples parallel to the direction of the muscle fibres, measuring 1 cm in height, 1 cm in width, and 2 cm in length, then

cut each sample. We shared the cube-like sample with the Volodkevitch bite jaw in the middle and placed it perpendicular to the longitudinal orientation of the fiber. We measured Warner-Bratzler's shear force in kilogrammes (kg) units, the average peak positive force of all subsample readings for each sample.

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Colour Attributes

The colour analysis involved the removal of the pectoralis major muscles from each treatment carcass, which were vacuum-packed and categorised into three ageing periods (1 day, 3 days, and 7 days) at 4°C. The meat colour was assessed using a ColourFlex spectrophotometer (Shenzhen 3nh Technology Company Ltd., China) to acquire the International Commission on Illuminance Lab values (L*: lightness, a*: redness, b*: yellowness). The D65 illuminant, with a 10° standard observer angle and a 5 cm aperture size, was employed for measurement according to the methodologies outlined by [17]. The gadget was calibrated using black and white tiles before examination. The frozen specimens from the -80°C freezer were defrosted overnight at 4°C. The thawed samples were unpacked and allowed to bloom for 30 minutes, after which the bloomed surface was positioned in contact with the base of the ColourFlex cup. After the initial measurement, the cup was turned 90° for each sample during the second and third readings. The mean of the three readings was utilised to denote the value of each colour parameter according to [17].

Data and Statistical Analysis

The study employed a completely randomised design (CRD) for its experimental design and data analysis. The current study utilised the GLM procedure (General Linear Model) of the Statistical Analysis System [18] software program version 9.1. The ANOVA procedure was used for the analysis of the results, identifying ageing and refrigerated storage (numbers 1, 3, and 5) as the main effects and refrigerated storage as the interaction. We used the Duncan Multiple Range Test to compare and separate the means where we observed significant differences. The significance level was set at $P \leq 0.05$.

Result and discussion

Microbiological Analyses and Oxidative Stability According to [9], microbial growth, lipid oxidation, meat safety, and quality were significantly affected by slaughter ageing, the transfer of contaminants during the slaughter process, maintaining temperature, and after storage. Also, [9] reported that refrigerated storage influenced the proliferation of microbes in meat and meat products. As shown in Table 1 below, results from the current study indicate an increase in microbial deterioration in broiler chicken meat as postmortem ageing increases from days 1, 3, and 5. On day 1, microbial counts showed a population decrease. However, on days 3 and 5 of postmortem ageing, a noticeable increase ($P \leq 0.05$) in total bacterial counts was observed in the various samples. The highest counts were in total aerobic bacteria, followed by Pseudomonas spp., Enterobacteriaceae, and lactic acid bacteria. Following refrigerated storage at 4ºC, total aerobic counts in chicken meat were 2.01, 2.94, and 2.61 Log10 CFU/g.

Pseudomonas spp., a gram-negative psychotropic bacterium often found at refrigerated temperatures, is considered the chief microorganism responsible for the spoilage of chicken meat during storage [19]. When the body was stored for 1, 3, or 5 days after death, acceptable levels of all microbes were found. These levels align with what [6] found. However, the total bacterial count in groups was within an acceptable range.

According to [20], lipid oxidation is the major non-microbial cause of spoilage in meat and meat products, primarily under pro-oxidative conditions such as storage. [21] also observed increased microbial following ageing and storage, aligning with the current work. Lipids and proteins are susceptible to oxidative changes because of the rapid depletion of endogenous antioxidants after slaughter [22] during refrigeration and frozen storage. Malondialdehyde (MDA) is one of the essential aldehydes produced during the secondary oxidation of polyunsaturated fatty acid lipids. It is considered a significant marker of lipid oxidation.

The thiobarbituric acid reactive substances (TBARS) assay for measuring malondialdehyde is the most common way to check for lipid oxidation in muscle because it is sensitive and easy to use [23]. Broiler chickens underwent postmortem ageing periods, which affected their muscles' TBARS value and carbonyl content. Postmortem aging on days 1, 3, and 5 resulted in significantly higher lipid oxidation concentration, carbonyl content, and lower free thiol content. In general, lipid oxidation values increased ($P < 0.05$) with storage time and are in line with the works of [24] in poultry, [25] in ostriches, and [26] in rabbits.

Nonetheless, the lipid peroxidation concentrations on days 3 and 5 were considerably ($P < 0.05$) elevated compared to day 1. Lipids show chemical volatility. Consequently, they are susceptible to oxidation, especially during postmortem and storage [27]. The present study did not attain the threshold value of TBARS (5 mg MDA/kg) for identifying off-odours and off-taste, [5] highlighted. Similarly, [12] reported that alterations in lipid oxidation result in off-odours, off-tastes, discolouration, protein breakdown, hazardous chemical buildup, and reduced shelf life, impacting consumer health.

As shown in Table 1, the postmortem aging period was significantly (P<0.05) affected by protein oxidation, as assessed by free thiol concentrations. The measurement of protein thiol groups indicated a decrease in thiol concentration from 41.61 to 37.49 nmol per mg of protein after postmortem storage. The deterioration of protein oxidation during refrigerated storage significantly reduced the protein thiols. According to [27], protein oxidation occurs when proteins interact with lipid hydroperoxides or secondary lipid oxidation products such as aldehydes, particularly the nitrogen or sulfur centers of reactive amino acid residues. The onset of lipid oxidation in meat and meat products appears to occur more rapidly than the oxidative destruction of myofibrillar proteins. Lipid-derived radicals and hydroperoxides are more prone to induced protein oxidation [28]. These findings align with those of [29] and [30] who demonstrated that free thiol and carbonyl groups markedly diminished and augmented with meat ageing.

Table 1 Effect of Ageing on Microbial Spoilage and Oxidative Stability of Pectoralis Major Muscle in Broiler Chickens

Parameter	Postmortem ageing periods		
	1d	3d	5d
Total aerobic count (Log10 CFU/g)	$2.01^{c} \pm 0.04$	$2.94^{b} \pm 0.09$	$3.61^{a} \pm 0.06$
Lipid oxidation (mg MDA/kg meat)	$1.34^{c} \pm 0.03$	$1.90^{b} \pm 0.06$	$2.76^{a} \pm 0.13$
Free thiol content (nmol/mg protein)	$41.61^{a} \pm 0.64$	$39.49^{b} \pm 0.046$	$37.49^{c} \pm 0.38$
Carbonyl content (nmol/mg protein)	$2.04^{c} \pm 0.09$	$2.79^{b} \pm 0.02$	$3.01^{a} \pm 0.09$

a,b,c, least square means with different superscripts in the same row indicate a significant difference in the ageing period at $p< 0.05$.

Myofibrillar Protein Profile (myosin and actin)

Table 2 below shows that ageing affects the breakdown of myosin and actin (reflective density/mm2) in the pectoralis major muscle of broiler chickens. The following concentrations were used to show the changes in the myosin heavy chain: 54.20c ± 0.50, 56.91b ± 0.39, 58.65a ± 0.28, and 18.66c ± 0.20, 19.28b ± 0.16, 19.83a ± 0.17 for actin, respectively. We measured the strengths and intensities of myosin-heavy chains and actin proteins by assessing each identified band's reflective density (RD) in the Myofibrillar Protein Profile (myosin and actin). Table 2 illustrates the impact of postmortem ageing periods on the degradation of myosin-heavy chains and actin in muscle.

The muscles of broiler chickens undergoing postmortem ageing periods showed an increased reflective density of myosin and actin-heavy chains. The ageing process significantly influenced myosin heavy chain values (P<0.05). Augmented oxidation can amplify protein breakdown by proteases [31]. According to [32], protein oxidation speeds up the breakdown of myosin-heavy chains and actin. Myosin is the primary protein in the myofibril complex, comprising about 45% of the total myofibrillar proteins in avian muscle tissues [33].

According to [34], the early oxidation of chicken myofibrils resulted in changes to myosin, particularly the intermolecular cross-linking of myosin-heavy chains and modifications of thiol groups in the myosin ATPase active site. Myofibrillar proteins are prone to oxidative processes, with myosin showing the highest sensitivity [35]. According to [31], Actin protein plays a crucial role in muscle contraction. [32] observed that actin breakdown is negligible in rabbits under refrigerated storage. It has been seen that actin bands stay mostly stable even in oxidative environments created by β-calpain [31] and chemical oxidation [36].

Table 2 Effect of Ageing on the Degradation of Myosin and Actin (reflective density/mm2) of Pectoralis Major Muscle in Broiler Chickens

Parameter	Post-mortem ageing periods		
	1d	3d	5d
Myosin heavy chain	$54.20^{c} \pm 0.50$	$56.91^{b} \pm 0.39$	$58.65^{a} \pm 0.28$
Actin	$18.66^{c} \pm 0.20$	$19.28^{b} \pm 0.16$	$19.83^{a} \pm 0.17$

^{a,b,c} Least square means with different superscripts in the same row indicate a significant difference in the ageing period at $p< 0.05$.

Table 3 demonstrates the effect of ageing on the pH of the pectoralis major muscle in broiler chickens during refrigeration at 4 ^{o}C. Overall, pH values substantially increased ($p< 0.05$) with prolonged storage duration. While ageing procedures did not influence ultimate pH ($p > 0.05$), day 1 displayed lower muscle pH values than day 3 and day 5. Irrespective of the treatment, the pH levels consistently increased over time. The buildup and proteolytic degradation of compounds resulting from bacterial activity on meat may explain the elevated pH during preservation [37]. [35] observed similar results when chilling chicken breast meat. The water holding capacity in drip and cooking losses of chickens treated with various

postmortem ageing durations. On the first, third, and fifth postmortem days, there was a substantial ($p<0.05$) rise in drip loss in the birds, although cooking loss decreased during the postmortem ageing periods.

Drip loss levels increased significantly ($p< 0.05$) with prolonged ageing. Previous research on meat from several animal species revealed that aging reduced meat's water-holding ability [38]; [39]. When exposed to external forces such as cutting, heating, grinding, and pressing, water-holding capacity refers to the meat's ability to retain intrinsic and extrinsic moisture. It is a crucial quality criterion in the chicken meat market, influencing palatability and economic factors [40].

Irrespective of the treatment group, water-retaining capacity significantly increased ($p<0.05$) over time. Age-related deterioration of collagens and myofibrillar proteins, which reduces the ability of myofibrillar proteins to retain water, may cause this outcome [41]. Tenderness is the paramount sensory attribute influencing consumer approval of meat [42] According to [43], aging affects meat tenderness due to intricate changes in muscle metabolism. From d 1 to d 5, the shear force values decreased significantly.

Other experiments [44; 45] showed similar results, suggesting postmortem ageing improves meat tenderness. The shear force values for postmortem ageing periods were substantially higher (1.13 kg on day 1 compared to 1.07 kg on day 3 and 1.06 kg on day 5). With age, the shear force values decreased significantly. During cold storage, enzymes may break down collagen-containing muscle fibres, which may cause intense activity and lower shear force measurements [46]. Postmortem alterations during ageing, which impact the muscle's contractile system or myofibrils, influence the ultimate tenderness of the meat [47; 48]. The length of time spent ageing affected the meat's lightness.

Table 3 demonstrates that postmortem ageing and refrigerated storage significantly impacted meat odour. The colour of the right pectoralis major muscle (L*, a*, and b*) showed significant differences on days 1, 3, and 5. The rise in L* value during storage corresponds with the findings of [49] and [42], which indicated that ageing enhanced muscle lightness.

In the current investigation, the meat's redness decreased with prolonged storage. This observation aligns with the findings of [50]. Redness is the primary colour criterion for evaluating meat oxidation [51]. This has to do with the breakdown of myoglobin into metmyoglobin. When low metmyoglobin-reducing activity (MRA), myoglobin breaks down into metmyoglobin, which causes methaemoglobin to build up in the meat [30], This alteration reduces the redness and renders the meat unappealing to consumers [52]. According to [53], lipid oxidation and TBARS values are associated with variations in a* values. On days 1, 3, and 5 postmortems, the meat exhibited significantly ($p<0.05$) increased lightness (L*) and reduced redness (a*) and yellowness (b*) values.

This can be attributed to the differences in pH values between different restraint methods. [53] discovered that the colour of breast meat is often associated with changes in postmortem muscle pH. [53] assert that breast muscles with a lower pH have a paler hue than those with a higher pH in broiler chickens. [54] identified a substantial positive association between redness and yellowness. [55] reported a correlation between meat's redness and yellowness, indicating that meat with elevated a* values typically has higher b* levels. Regardless of the postmortem ageing periods, lightness values increased ($P<0.05$), whereas redness and yellowness values decreased ($P<0.05$) with extended postmortem ageing. Myoglobin oxidation during ageing may significantly reduce color attributes, as it is the primary hemoprotein responsible for meat colouration [56].

Table 3 Effect of Ageing on Muscle pH, Drip Loss (%), Cooking Loss (%), Warner-Bratzler Shear force (kg), and Colour Characteristics in Pectoralis Major Muscle of Broiler Chickens

Parameter	Postmortem ageing periods		
	1d	3d	5d
pH (unit)	$5.30^{b} \pm 0.03$	$5.38^{bb} \pm 0.04$	$5.67^{a} \pm 0.04$
Drip loss (%)	$1.62^{c} \pm 0.06$	$2.21^{b} \pm 0.04$	$2.81^{a} \pm 0.06$
Cooking loss(%)	$28.97^{a} \pm 0.24$	$27.41^{b} \pm 0.30$	$25.45^{c} \pm 0.32$
Warner-Bratzler Shear force (kg)	$1.13^{a} \pm 0.01$	$1.07^{bb} \pm 0.00$	$1.06^{b} \pm 0.01$
Lightness (L*)	$61.24^{c} \pm 0.41$	$62.9^{b} \pm 0.50$	$64.87^{a} \pm 0.49$
Redness (a*)	$5.95^{aa} \pm 0.09$	$5.80^{ab} \pm 0.16$	$5.53^{bb} \pm 0.10$
Yellowness (b*)	$7.73^{a} \pm 0.19$	$6.95^{b} \pm 0.13$	$6.26^{c} \pm 0.11$

a,b,c, least square means with different superscripts in the same row indicate a significant difference in the ageing period at $p< 0.05$.

Conclusion

In summary, the microbial counts, lipid oxidation, heavy chains, myofibrillar fragmentation index, and the physicochemical properties of the meat, including its colour, muscle ph, water-holding capacity, shear force, drip loss, and cooking loss, significantly influence the ageing and refrigerated storage of broiler chicken meat. Over time, microbial populations tend to increase with increasing storage periods due to the proliferation of bacteria, which often leads to spoilage. Though refrigeratored storage slows down, it does not wholly prevent the growth of the microbes. Following prolonged ageing, lipid oxidation rises, producing off-flavours and reduced nutrient contents as fats degrade into other secondary oxidative products. Physicochemical properties, such as color, muscle pH, water-holding capacity, shear force, drip loss, and cooking loss, change as the meat ages. In general, the muscle pH of meat decreases due to the accumulation of lactic acid

from postmortem glycolysis, which increases texture values, resulting in tougher meat. Lastly, improved refrigerated storage and meat ageing are vital to ensuring better meat quality, safety, and shelf life.

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تأثير التخزين البارد على أكسدة الدهون والبروتينات والتلف الميكروبي والخصائص الفيزيائية والكيميائية للحوم الدجاج.

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الخلاصة
هدفت هذه الدراسة الى دراسة على أكسدة الدهون والبروتينات في لحم الدجاج، والتحلل الميكروبي، والصفات الفيزيائية والكيميائية أثناء التبريد. شملت الدراسة 30 دجاجة لاحم *ROSS* عمرها 42 يومًا وبمعدل وزن 2.023 ± 0.01 كغم في مصنع تجاري في أربيل، إقليم كردستان شمال العراق. بعد النزيف وإزالة الأحشاء، تم تقسيم عينات عضلات صدور الدجاج إلى ثلاثة أجزاء ثم تم وضع ترقيمها وإغلاقها وتخزينها في ثلاجة عند 4 درجات مئوية لمدة 1 و3 و5 أيام. في هذه الدراسة، كانت هناك زيادة في أنشطة الميكروبات، بما في ذلك بكتيريا حمض اللاكتيك، والبكتيريا المعوية، وأنواع الزائفة، والتعداد الهوائي الإجمالي ($P < 0.05$) مع ارتفاع أيام التعتيق. تغيرت المواد المتفاعلة لحمض الثيوباربيتوريك والكربونيل والثيول الحر بشكل ملحوظ مع عمر اللحم لمدة 1 و 3 و 5 أيام. وبالمثل، فإن تخزين لحوم الدجاج اللاحم والتخزين المبرد فيها يؤثر على اللون، ودرجة الحموضة العضلية، ونسبة *WHC*، وفقدان التنقيط، وفقدان الطهي، والسلاسل الثقيلة (الأكتين والميوسين). يعد تحسين التخزين المبرد وتعتيق اللحوم أمرًا حيويًا لضمان جودة أفضل للحوم وسلامتها ومدة صلاحيتها.

الكلمات المفتاحية: الأكسدة، الفساد الميكروبي، جودة اللحوم، لحم الدجاج، والتخزين بالتبريد.