ZANCO Journal of Pure and Applied

TYPE Original article
DOI http://dx.doi.org/10.21271/ZJPAS.37.3.11

OPEN ACCESS

*Corresponding author
Naska Abdulqadir Mohammed
naska.muhamad@univsul.edu.iq

RECEIVED :31 /01 /2025
ACCEPTED :24/03/ 2025
PUBLISHED :30/ 06/ 2025

KEYWORDS:
Broiler meat, frozen, nutritional value, re-frozen, lipid-protein oxidation.

The Impact of repeated cycles of freezing, thawing, and refreezing on nutritional value and lipid-protein oxidation in broiler breast meat

Bestoon Hassan Ahmed[1] and Naska Abdulqadir Mohammed[2*]

1Department of Animal Resources , College of Agricultural Engineering Sciences, Salahaddin University- Erbil, Erbil, Kurdistan Region, Iraq

2Department of Food Science and Quality Control, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Iraq.

ABSTRACT

The objective of this study was to assess the impact of freezing and refreezing temperatures, as well as the duration of frozen storage, on fatty acid and amino acid profiles, and the oxidative stability of lipids and proteins in broiler breast meat. Forty broiler breast muscle samples were divided into two primary groups. The first group was subdivided into three equal sections, each subjected to freezing durations of 0, 30, 60, and 90 days. The second group underwent freezing cycles for 30, 60, and 90 days, followed by overnight thawing at 4 °C. Refreezing intervals of 0, 30, 60, and 90 days were then implemented before the meat quality assessment. The results showed a significant reduction ($P < 0.05$) in essential amino acids, such as lysine, threonine, and valine, in broiler breast meat samples. These reductions were observed after 30, 60, and 90 days of freezing in the first group and following two or three cycles of freezing and refreezing in the second group. Non-essential amino acids such as tyrosine and arginine showed no significant reduction during all refreezing intervals. Fatty acids exhibited a significant decline ($P < 0.05$), including saturated and monounsaturated fatty acids, but linoleic acid remained stable across all storage durations.Lipid and protein oxidation levels increased steadily over time, particularly during prolonged storage and repeated freeze-thaw cycles. These findings indicate that extended freezing and refreezing negatively affect the amino acid and fatty acid profiles, contributing to a decline in broiler meat quality.

1.Introduction

Breast meat derived from broiler chickens is widely recognized as a result of its rich protein profile and low levels of fat, making it a vital component of a nutritious diet (Abdulla *et al*., 2019). With growing consumer interest in healthy poultry options, the evaluation and management of broiler meat quality has become increasingly important (Carvalho *et al*., 2015). This meat is not only a great source of high-quality proteins, but also provides essential microelements necessary for maintaining good health throughout life (Biesalski, 2005). Proteins are formed through a process called protein synthesis, in which amino acids are linked in long chains. These chains then fold and coil into specific shapes, creating the required protein structures. Proteins are composed of amino acids connected by peptide bonds (Macelline *et al*., 2021). Since our bodies cannot produce all the essential amino acids on their own, having a diverse amino acid profile is crucial (Alina and Ovidu, 2007). Nutritional assessment of meat depends heavily on amino acids. To maintain health, it is crucial for both humans and animals to ingest essential fatty acids that their bodies cannot synthesize on their own (Hou *et al*., 2015). The proper functioning of all human tissues is significantly dependent on the presence of polyunsaturated fatty acids, such as total n-3 (omega-3) and n-6 (omega-6) (Mititelu *et al*., 2024).While meat serves as a significant source of essential nutrients for humans, it has a relatively short shelf-life and can quickly spoil owing to factors such as protein degradation, lipid oxidation, enzyme activation, and microbial growth (Aladdin *et al*., 2022;Rahman, 2023). Various preservation methods are often employed to increase the preservation period of fresh meat (Hammad *et al*., 2019). However, prolonged freezing and repeated freeze-thaw cycles are associated with significant declines in meat quality over time, primarily due to structural damage in muscle cells and oxidative changes in lipids and proteins (Leygonie *et al*., 2012; Coombs *et al*., 2017). While previous studies have examined the physical and biochemical changes that occur during freezing and storage, limited research has focused on the combined effects of freezing and refreezing on the fatty acid and amino acid composition, as well as lipid and protein oxidation. Addressing this gap is particularly important, as lipid and protein oxidation not only compromise meat quality but may also have health implications for consumers (Zhang and Ertbjerg, 2019; Farhan *et al*., 2024). Therefore, further investigation is necessary to better understand how freezing and refreezing processes influence broiler meat at the molecular level and its overall quality (Saber, 2023).

Freezing meat at temperatures between -18°C and -20°C is the standard for preserving its quality to closely resemble fresh meat. Nevertheless, thawing and subsequent refreezing can weaken the structural integrity of muscle cells, causing moisture migration, oxidative degradation, and significant declines in both nutritional and physical quality attributes (Soyer *et al*., 2010; Charoenrein, 2018). While freezing has been widely studied, few studies have explored how repeated freezing and refreezing exacerbate oxidative changes in lipids and proteins, contributing to these quality losses. This study aimed to investigate the impact of freezing and refreezing on the fatty and amino acid profiles and the oxidative stability of lipids and proteins in broiler breast meat.

2. Material and methodology:

Approximately 40 broiler breast meat samples were collected from Taza and Ehtimad slaughterhouses located in Erbil. The samples were subsequently transported and packaged in hygienic polyethylene bags to the laboratory. Broiler breast samples were separated into two groups according to different treatment processes. The initial group was split into three equal sections, each section having an equal weight, and exposed to different freezing durations: One sample remained at 0 days (control group), while the others were frozen for 30, 60, and 90 days. The second group underwent freezing for 30, 60, and 90 days at -20°C. The process involved thawing for 12 hours at 4°C, followed by refreezing. The treated samples were maintained at -20°C for 30, 60, and 90 days prior to thawing and testing. After freezing, the samples underwent a thawing process at 4°C for approximately 12 hours

overnight, maintaining their original packaging throughout, and were then refrozen at -20°C for the same length of time. Both treatment groups underwent a comprehensive evaluation of the physical attributes and nutritional qualities of the broiler chicken meat.

2.1 Amino Acid Profile

An amino acid analyzer was used to determine the amino acid content of meat (Sabagh et al., 2016). Briefly, the sample was defatted using diethyl ether and 0.4 g was transferred into a sealed test tube for hydrolyzation using 6 N HCl (approximately 5 mL) at 115°C for 24 h. All test tube contents were then transferred to containers for HCl evaporation at 55°C in a water bath. All containers received an additional 5 mL of distilled water, which was evaporated to remove HCl. Finally, the samples were dried to obtain dry films. After filtering the solution via a 0.45 µ membrane filter, 2 mL of buffer (0.1 N sodium acetate buffer, pH 2.2) was used to dissolve the resulting dry film. The materials were stored at -80°C in glass vials until the amino acids were fractionated using an amino acid analyzer.

2.2 Fatty Acid Profile

The fatty acid profiles were determined using the method described by (Kaczmarek and Muzolf-Panek , 2021). Fat was extracted from meat samples using a chloroform-methanol solution at a volume ratio of 2:1. Following separation of the upper phase, the chloroform present in the samples was evaporated. The treated samples were subsequently preserved at a temperature of -20°C until they underwent analysis by gas chromatography (GC). A gas chromatograph 7890 B series (Agilent, USA) was used for the separation of fatty acids. This instrument was fitted with a BPX-70 capillary column, measuring 60 m in length, 0.22 mm in internal diameter, and featuring a film thickness of 0.25 mm, along with a flame ionization detector. The operating conditions specified the use of helium as the flow rate of the carrier gas was set to 30 cm/s.The detector was set to 260°C, and the temperature of oven was gradually increased starting from 50°C to 250°C at a rate of 10°C/60 s, kept constant for 25 min. The separation of fatty acids was identified by comparing their retention times with those of the standard solutions. The peaks observed in the chromatogram were used to evaluate the fatty acid profiles, The total derivatives of fatty acid methyl esters are indicated in terms of gper 100 g of dry matter.

2.3 Mineral Profiles

The Breast meat samples were manually divided into smaller pieces (3×3cm), homogenized (Bio-Gene, USA), and stored in polyethylene bottles at 4 °C. All the samples were lyophilized. Finally, the samples were dried using liquid nitrogen and preserved at -80 °C until analysis. Each sample was weighed and separated into three digestion flasks for mineral analysis, followed by the addition of 10 mL nitric acid, which was heated in a fume chamber until no red nitrogen dioxide was produced. The flasks were cooled before each flask received 4 mL 70% perchloric acid ($HClO_4$). The mixture was then heated again to dry contents. After digestion, each sample was diluted to a volume of 50mL.The absorbance was measured using an atomic absorption spectrophotometer and compared to a blank. The concentration of each mineral was recorded, and the overall mineral concentration in mg/100 g was determined as follow (Akinnusi et al., 2018):

Total mineral concentration (mg/100 g) = {[concentration (mg/L) ×Dilution Factor (L)]/ weight of the sample (g)} ×100.

2.4 Malondialdehyde assay (MDA):

The measurements were conducted following the method outlined by (Abubakar et al., 2021). Initially, a total of approximately 1 g of muscle samples was blended with 4 mL of a solution containing 0.1 mM BHT and 0.15 M KCl. The Next procedure involved mixing 200 µL of the homogenate with a solution of thiobarbituricacid reactive substances (TBARS) and heating the combination at 95°C for 60 min in a water bath, which resulted in a pink coloration of the solution. After cooling, one (1) milliliter of water in the form of distilled and three (3) milliliter of butyl alcohol (n-)were combined and blended carefully. The blend was then placed in a centrifuge and rotated at 5000 rpm for 10 min. The absorbance of the supernatant was evaluated using a spectrophotometer at 532 nm (Beckman, Domont, France), with an appropriate blank for comparison. The amount of secondary lipid

oxidation in the form of malondialdehyde present in the breast muscle was assessed as MDA per mg of muscle tissue, and the value of TBARS was derived from a standard curve based on 1,1,3,3-tetraethoxypropane.

2.5 Quantification of muscle thiols

Muscle thiols were quantified using a fluorometric assay kit provided by Abcam (Cambridge-USA). First, a standard solution of glutathione was prepared a standard solution of glutathione, along with a 100x green thiol indicator, and all necessary dilutions were prepared in accordance with the guidelines provided by the manufacturer. Subsequently 50 µL of the standard solution, blank, and muscle samples were added to the wells of a micro plate. The micro plate was incubated at room temperature for one hour, away from light. Following the incubation period, 50 µL of the prepared working solution was introduced into every well. Ultimately, an Agilent device from Germany was employed to monitor the increase in fluorescence at the wavelengths of 490/520 nm. The quantification of thiol content in breast muscle samples was achieved by plotting a standard curve.

2.6 Assessment carbonyl of levels in muscle tissue

A colorimetric assay kit from Abcam (USA)was used to assess the level of carbonyls in the muscle tissue. These processes involve several straightforward steps. First, the muscle samples were homogenized in distilled water to achieve a protein concentration of approximately 10 mg/ml.Next, 100 Micro liters(µL) of DNPH solution was added to each sample. The samples were vigorously shaken and incubated at room temperature for 10 min. Following this, 30 µL of TCA was added to each sample, mixed them thoroughly using a vortex, and then the samples were chilled on ice for five minutes. The samples were then centrifuged at maximum speed for two minutes. Following the removal of the supernatant, we added 500 µL of cold acetone to wash the pellets thoroughly. Afterward, each tube received an addition of 200 µL of guanidine solution, which and sonicated to ensure comprehensive mixing. Ultimately, the absorbance of light in optical contexts was measured using a microplate reader operating at a wavelength of 375 nm, and a standard curve was plotted to determine the carbonyl content for each individual sample.

2.7 Muscle myoglobin measurement

Muscle myoglobin concentrations were assessed using an enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, USA). First; we prepared 100 µL of homogenized muscle samples and added 100 µL of standards to the wells of a microplate. For control purposes, we also included 100 µL of PBS in separate wells. Subsequently, we incorporated 10 µL of a balanced solution into the muscle samples and thoroughly mixed the entire mixture. Following the addition of 50 µL of conjugate to each well, with the exception of the blank wells, the samples were subsequently incubated at 37°C for duration of one hour.Once the incubation was complete, the microplate was washed 50 µL of stop solution was added, and approximately 50 µL of substrate A and 50 µL of substrate B were add to each well. Prior to measuring the optical density with a microplate reader at 450 nm (Awareness Stat Fax 2100, USA), the samples were incubated again for 20 min at 37°C.

2.8 Statistical analysis

A completely randomized design (CRD) was utilized in the formulation of the experiment. The statistical analysis was performed using the General Linear Models (GLM) approach provided by the Statistical Analysis System (SAS institute,2001). software, Version 9.2. In instances where significant effects were detected,Duncan's multiple range tests were proceeded to compare the means. the level of statistical significance was determined to be $p<0.05$

3. Result and discussion

Table 1. Impact of storage conditions (mean ± standard error) and storage methods on the amino acid content in the form of essential and non-essential (grams per each 100 grams of dry matter) chicken breast meats. [

Treatment / Period	Fresh	Frozen			Refrozen		
Amino acids	**24 hour**	**30 days**	**60 days**	**90 days**	**30 days**	**60 days**	**90 days**
Isoleucine	4.120 ± 0.07a	4.033 ± 0.07a	3.900± 0.11a	3.826 ± 0.09a	3.316 ±0.11b	3.240 ± 0.06b	3.033 ± 0.07b
Leucine	6.010 ± 0.06a	5.796 ± 0.05a	5.580 ± 0.05b	5.303 ± 0.09bc	5.126 ±0.02cd	4.856 ± 0.04d	4.346 ± 0.12e
Lysine	6.073 ± 0.02a	5.993 ± 0.07a	5.700 ± 0.01b	5.436 ± 0.06b	5.010 ± 0.13c	4.863 ±0.05cd	4.673 ± 0.07d
Methionine	2.453 ± 0.02d	2.306 ± 0.03d	2.126 ± 0.10c	2.115 ± 0.05a	2.083±0.03ab	1.886 ± 0.02cb	1.630 ± 0.07c
Phenylalanine	3.530 ±0.03b	3.436 ± 0.09a	3.950 ± 0.06a	3.126 ± 0.02c	3.026 ±0.06cd	2.800 ± 0.05d	2.546 ± 0.05e
Threonine	5.503 ± 0.06a	5.433 ± 0. 01a	5.375 ± 0.04a	5.373 ± 0.13a	4.806 ±0.07b	4.670 ± 0.05b	4.173 ± 0.02c
Valine	4.556 ± 0.02a	4.410 ± 0.05a	4.200 ± 0.08b	3.970 ± 0.04c	3.433 ±0.11d	3.276 ± 0.02de	3.073 ± 0.03e
Glutamine	13.876±0.09a	13.703±0.07ab	13.365±0.04b	13.343±0.04b	11.850±0.11c	11.736±0.06cd	11.340±0.05d
Tyrosine	3.033±0.02a	2.880±0.02ab	2.760±0.02ab	2.720±0.02ab	2.430±0.09a	2.256±0.02ab	1.360±0.06 b
Arginine	4.713±0.02a	4.550±0.03b	4.155±0.03c	4.136±0.06cd	4.093±0.04d	4.026±0.02ed	3.913±0.02e
Alanine	4.546±0.12^{cab}	4.530±0.07^{cab}	4.500±0.05^{cb}	4.873±0.06^{ab}	4.283±0.02^{c}	4.263±0.02^{ab}	4.193±0.04^{cab}
Serine	3.840±0.06^{a}	3.750± 0.03^{ab}	3.725±0.01^{b}	3.676±0.01 ^{b}	3.476± 0.02^{c}	3.290±0.01^{d}	3.203±0.04^{d}
Glycine	5.363±0.03^{a}	4.886±0.03^{b}	4.325±0.03^{c}	4.433±0.02^{fe}	4.163±0.02^{de}	3.926±0.03^{f}	3.920±0.02^{f}
Aspartic acid	8.766±0.08^{e}	8.563±0.04^{d}	8.435±0.05^{a}	8.330±0.06^{b}	8.283±0.08^{bc}	8.173±0.02^{b}	8.140±0.02^{c}
Cysteine	0.504 ±0.01 ^{a}	0.471±0.01 ^{b}	0.405±0.01 ^{c}	0.323±0.01 ^{d}	0.290±0.01 ^{e}	0.276±0.01 ^{e}	0.244±0.01 ^{f}
TOTAL AMIN ACIDS	76.886±0.03^{a}	74.74 ±0.08^{a}	72.501 ±0.01^{a}	70.983±0.12^{b}	65.668±0.10^{c}	63.537 ±0.05^{d}	59.787±0.06^{e}

*a- fThe Variations are indicated by distinct letters in the same row (P < 0.05).

3.1Amino acid profile in broiler-breast-meat

The amino acid levels measured in grams per 100 g of dry broiler breast meat subjected to both freezing and refreezing after 30, 60, and 90 days are shown in **Table 1**. A significant decrease in essential amino acids was observed (P≤0.05) in samples of breast broiler meat after storage under freeze and refreeze conditions for 30, 60, and 90 days. However, the amino acids isoleucine (ILEU) and threonine (THR) showed non-significant decreases after 30, 60, and 90 days. Similarly, leucine (LEU), lysine (LYS), phenylalanine (PHE), and valine (VAL) also showed non-significant declines at the 30-days mark compared to the fresh samples. While the concentration of methionine increased with a lengthened storage duration of freeze and refreeze cycles in comparison with fresh samples, this increment may be due to the increased activity of bacteria after thawing and refreezing, which causes changes in the bioavailability of amino acids by using some amino acids originating from both alimentary and endogenous proteins (Adedokun *et al*., 2014).In addition, the total amount of essential AAs in the frozen and refrozen breast broiler samples declined considerably (P≤0.05), except at 30 days of freezing, which declined insignificantly when compared with the quantity of AAs in the

control group (fresh sample). Regarding the effects of freezing and refreezing durations on non-essential amino acids, the results indicated that TYR and ARG showed non-significant reductions at 30, 60, and 90 days, except for TYR at the 90-days refreezing point. In contrast, GLY, ASP, and SYS significantly decreased (P≤0.05) at 30, 60, and 90 days under both frozen and refrozen storage conditions. Similarly, there was a notable decrease (P≤0.05) in GLU and ALA levels with the lengthening of frozen and refrozen storage durations, with the exception of the 30-day frozen storage, relative to fresh breast broiler samples that were not frozen. The total amount of non-essential AAs in the refrozen breast broiler samples decreased considerably (P≤0.05), whereas it decreased insignificantly through the extension of the frozen storage period compared with the quantity of AAs in the control group (fresh sample). These findings indicate that both essential and non-essential amino acids decreased during extended periods of frozen and refrozen storage. This decline may be linked to increased drip loss, which could contribute to the overall reduction in amino acids and the denaturation of these compounds during freeze-thaw and refreeze cycles. An additional consideration could be the development of ice crystals, which may interfere with the integrity of muscle tissue, leading to the breakdown of muscle fibers and oxidation of proteins. Additionally, a group of enzymes known as hydrolyses, which become active after thawing, may further reduce the concentration of amino acids in their free form. The results obtained in the current study align with the findings of Mohammed *et al*. (2021); they revealed that the concentrations of certain essential amino acids, including histidine (HIS), isoleucine (ILEU), leucine (LEU), lysine (LYS), and methionine (MET), along with all non-essential amino acids, decreased in chicken samples after 9.0 months of storage when compared to the control samples, although during the 9.0 months of storage in the freezer and re-frozen storage, PHE, THR, TRY, and VAL demonstrated an increase in levels compared to the control group.

Table 2. Impact of storage conditions (mean ± standard error) and storage methods on fatty acid composition in the form of saturated and unsaturated (grams per each 100 grams of dry matter) broiler breast meats

Treatment / Period	Fresh	Frozen			Refrozen		
Fatty acids	**24 hour**	**30 days**	**60 days**	**90 days**	**30 days**	**60 days**	**90 days**
Myristoleic	0.193 ± 0.01a	0.170 ± 0.01b	0.135 ± 0.01c	0.113 ± 0.01cd	0.093±0.01ed	0.083± 0.01e	0.056± 0.01 f
Pentadecanoic	0.056 ±0.01ab	0.055 ±0.01ab	0.053 ±0.01ab	0.046 ± 0.01cb	0.036±0.01cd	0.030± 0.01d	0.026± 0.01 d
Palmitoleic	6.086 ± 0.04a	5.686 ± 0.01b	5.455 ± 0.03c	5.210 ± 0.01 d	5.063± 0.03e	4.876± 0.01f	4.726± 0.02 g
Stearic	5.530 ± 0.04a	5.416 ± 0.01b	5.225 ± 0.03c	5.100 ± 0.01 d	4.923±0.02e	4.816± 0.01f	4.610± 0.02g
Oleic	37.743 ±0.44a	37.150±0.03ab	37.170±0.06ab	36.850±0.15cb	36.826±0.06cb	36.516±0.06cb	36.246± 0.07c
Myristic	0.733± 0.03ab	0.666± 0.03cb	0.600 ± 0.01c	0.566 ± 0.03c	0.400±0.01d	0.366± 0.03 d	0.233± 0.03 e
Linoleic	11.150 ±0.13a	10.943 ± 0.02a	10.645 ± 0.01a	9.726 ± 0.08a	9.430± 0.06a	8.740± 0.07a	8.086± 0.08a
Palmitic	27.826 ±0.34a	27.276 ±0.51cb	25.975 ± 0.35 d	24.046 ±0.39d	22.906±0.41de	22.506±0.26e	19.316± 0.40 f
Linolenic (C18:3 n-3)	0.623± 0.03 a	0.593± 0.01 a	0.475 ± 0.01 b	0.393 ± 0.01 c	0.280± 0.02 d	0.220± 0.01 de	0.156± 0.02 e
Eicosenoic	0.336 ± 0.03 a	0.293± 0.01 ab	0.275 ± 0.01 ab	0.270 ± 0.01ab	0.236±0.01cb	0.180± 0.01 cd	0.153± 0.02d
Linolenic (C18:3 n-6)	0.086 ±0.01ab	0.080 ± 0.01 a	0.070 ± 0.01 ab	0.066 ± 0.01 b	0.046± 0.01 c	0.038± 0.01 c	0.026± 0.01 d

Eicosadienoic	0.306 ±0.01ab	0.320 ± 0.01 a	0.300 ± 0.01 ab	0.270 ± 0.01cb	0.240± 0.02 c	0.186± 0.01 d	0.156± 0.01 d
Heptadecanoic	0.153± 0.01a	0.146 ± 0.01 a	0.135 ± 0.01 ab	0.116± 0.01 cb	0.113±0.01 cb	0.103± 0.01 cd	0.080± 0.01 d
Eicosatrienoic	0.173±0.01 a	0.166 ± 0.01 ab	0.146 ± 0.01 cb	0.145 ± 0.01cb	0.130± 0.01 c	0.103± 0.01 d	0.096± 0.01 d
Arachidonic	0.593± 0.05ab	0.603± 0.01 a	0.515 ± 0.02 cb	0.483±0.01c	0.373± 0.02 d	0.360± 0.02 d	0.303± 0.01 d
{Total fatty acid g/100 gram of dry matter}	91.587± 0.51a	89.563 ± 0.52 a	87.174 ± 0.03 a	83.13 ± 0.57 a	81.095 ± 0.46a	79.123 ± 0.27a	74.269 ±0.43 a

*a-eThe Variations are indicated by distinct letters in the same row (P < 0.05).

3.2Fatty acid profile in broiler breast meat

The variations in the fatty acid profiles of broiler breast samples throughout the freeze-thaw-refreeze cycles are shown in (**Table 2**). By prolonging the duration of refreezing storage periods to 30, 60, and 90 days for broiler breast samples, the fatty acid contents decreased considerably (P≤0.05), with the exception of linoleic acid, compared with the untreated sample (control). The levels of saturated fatty acids, such as (stearic acid C18:0) decreased significantly (P≤0.05) during extended frozen and refrozen storage. Similarly, the content (Pentadecanoic C15:0, Palmitic C16:0, Myristic C14:0, and Heptadecanoic C17:0) decreased significantly for some frozen storage durations and was insignificant for other extended frozen storage periods. Similarly, extending freezing and refreezing storage periods caused a significant (P≤0.05) decrease in the total saturated fatty acid content, excluding 30 days of freezing storage, compared to fresh samples (control). Mono-unsaturated fatty acids (Myristoleic C14:1, Palmitoleic C16:1, and Oleic C18:1 cis), increasing the numbers of cycles of freezing, thawing, and refreezing, resulted in a significant reduction in fatty acid content, except for Eicosenoic C20:1 at 30 days of freezing when compared with fresh samples.In addition, the amount of total mono-unsaturated fatty acids decreased significantly with prolonged frozen and refrozen storage.Finally, polyunsaturated fatty acid contents, including Linolenic C18:3 n-3, Eicosatrienoic C20:3, and Arachidonic C20:4, decreased significantly with extended frozen and refrozen storage, except at 30 days, when they declined insignificantly. Linolenic C18:3 n-6 and Eicosadienoic C20:2 decreased significantly (P≤0.05), except after 30 and 60 days of storage. with respect to the total polyunsaturated fatty acids in broiler chicken breast samples, prolonged freezing and refreezing durations caused a significant reduction in the content of total unsaturated fatty acids, with the exception that the content at 30 days of freezing appeared to be reduced non-significantly compared with fresh broiler chicken breast samples. The results of our study are consistent with those reported by Wu *et al*. (2021), who noted a significant reduction (P≤0.05) in the fatty acid content of meat as the frequency of freeze–thaw cycles increased. With an increase in the number of freeze-thaw cycles, there was a notable decline in the concentrations of saturated,mono-unsaturated, and poly-unsaturated fatty acids. During frozen and refrozen storage, fatty acid oxidation and breakdown of phospholipids and triglycerides occur. Lipid oxidation is the primary process, followed by lipid hydrolysis. The observed reduction in fatty acids in the form monounsaturated, polyunsaturated and saturated may be attributed to the elevated enzymatic degradation (hydrolysis) during freeze-thaw-refreeze storage. This is because lipolytic enzymes remain active, even when the product is frozen. Additionally, freeze-thaw cycles can accelerate the release of these enzymes due to ice crystal formation and cell membrane damages (Wu *et al*., 2021).

Table3. Impact of Freeze-Thaw-Refreeze Cycles on Mineral Content (mg/100 g of dry meat) of broiler chicken breastmeat (Mean ±Standard Error).

Treatment / Period	Fresh	Frozen			Refrozen		
Mineral content mg/100 g	**24 hour**	**30 days**	**60 days**	**90 days**	**30 days**	**60 days**	**90 days**
Calcium	5.986 ± 0.05 a	5.973 ± 0.03 a	5.963 ± 0.04 a	5.960 ± 0.02 a	5.951 ± 0.05 a	5.946 ± 0.03 a	5.934 ± 0.02 a
Manganese	2.066 ± 0.08 a	2.000 ± 0.08 ab	1.933 ± 0.10 a	1.800 ± 0.05 abc	1.666 ± 0.06 bcd	1.566 ± 0.12 cd	1.500 ± 0.05 d
Sodium	73.766 ± 0.58a	72.233 ± 0.68ab	71.933 ± 0.92 bc	70.536 ± 0.36 cd	68.976 ± 0.19 d	65.303 ± 0.23 e	60.100 ± 0.15 f
Copper	5.040 ± 0.03a	4.976 ± 0.07 ab	4.800 ± 0.09 bc	4.670 ± 0.04 c	4.456 ± 0.03 d	4.336 ± 0.03 d	4.116 ± 0.06 e
Zinc	2.983 ± 0.07 a	2.966 ± 0.15 a	2.870 ± 0.01 a	2.850 ± 0.17 a	2.476 ± 0.09 b	2.000 ± 0.05 c	1.663 ± 0.12 c
Magnesium	21.166 ± 0.17ab	20.866 ± 0.12ab	20.766 ± 0.20 a	20.535±0.70ab	20.366 ± 0.43 ab	20.233 ± 0.29 ab	20.000 ± 0.20 b
Potassium	320.523 ± 2.46a	318.093 ± 0.48a	316.100 ± 1.04ab	313.150±1.76bc	311.200 ± 1.42 cd	310.187±0.92 cd	307.087 ±1.62d
Phosphorus	263.596 ± 4.36a	255.476±3.18bc	248.353 ± 1.57 c	238.710 ±1.81d	259.630 ± 0.98 ab	257.993±1.94 ab	249.430 ±0.53c
Selenium	0.450 ± 0.02a	0.443 ± 0.01a	0.436 ± 0.01 a	0.413 ± 0.01 ab	0.420 ± 0.02 ab	0.376 ± 0.01 bc	0.346 ± 0.01 c
Iron	0.581 ± 0.02a	0.552 ± 0.01a	0.531 ± 0.01 a	0.519 ± 0.01 ab	0.497 ± 0.01 ab	0.443 ± 0.02 bc	0.395 ± 0.02 c

*a-f The variations are indicated by different letters located in the same row (P <0.05).

3.3Mineral profile in broiler breast meat

The changes in mineral concentrations in broiler breast meat due to freezing and refreezing are shown in (**Table3)**. The minerals {calcium (Ca), manganese (Mn), sodium (Na), copper (Cu), zinc (Zn), magnesium (Mg), potassium (K), phosphorus (P), selenium (Se), and iron (Fe)} were discovered in all breast broiler samples that were examined. Samples of breast broiler meat revealed that K was the most prevalent element during freezing and refreezing. The conclusions of the present study are consistent with those of Majewska *et al*. (2009), who determined that the most abundant minerals in both chicken and ostrich meat wear potassium and phosphorus. Additionally, (Chen ,2024) Discovered that K and phosphorus are two of the most abundant elements. The Ca and Mg contents in broiler breast meat decreased slightly (P>0.05) with extended storage periods (freezing and refreezing) compared to the samples in the control group (fresh samples).. Similarly, the amounts of Mn, Zn, K, Se, and Fe declined insignificantly during frozen storage only, whereas the quantity of these elements (Mn, Zn, K, Se, and Fe) declined (P≤0.05) with prolonged refreezing storage, excluding the Fe and Se contents, which decreased insignificantly at 30 days of refreezing storage when compared to fresh samples (without freezing).

Likewise, the results revealed that Na and Cu were reduced significantly(P≤0.05) after 60 and 90days of freezing and 30, 60, and 90days of refreezing storage compared to the control treatment. However, frozen storage led to a small decrease in phosphorus values; nevertheless, the content of P decreased significantly during refrozen storage. Finally, the results in Table 3

show that there was a slight increase in both P and Se values at 30days of refreezing storage compared to their values at 30, 60, and 90days of freezing. The reduction in the concentration of most minerals can be attributed to drip loss and dehydration, which are associated with freezing and refreezing storage. Microbial activity may be responsible for the observed reduction or increase in mineral content in broiler breast samples, which may affect the bioavailability of several minerals of either consumption or mineral origin (Viveros et al., 2002). (Bida,2019) and Mohammed et al. (2021) arrived the same conclusion.

3.4 Lipid oxidation malondialdehyde (MDA) in broiler chicken breast meat

Figure (1) shows that storing raw meat in a frozen significantly reduces lipid oxidation. However, cold temperatures do not completely inhibit the activity of certain enzymes in muscle, such as lipases and phospholipases. When water freezes, it increases the surface area where lipids and oxygen can interact, in unfrozen water, some chemical reactions may still occur. The degree of lipid oxidation in frozen raw meat varies based on several factors, with longer storage times tending to increase oxidation (Park et al., 2007). In the presences of free radicals, they react with unsaturated fatty acids, leading to the formation of peroxides and other primary products (Abdulla et al., 2019). As oxidation progresses, secondary products such as ketones, esters, and aldehydes can develop, causing meat to become rancid (Wu et al., 2021).

MDA is an important secondary product used to measure lipid oxidation. The concentration of (MDA) significantly increased (P≤0.05) when chicken breast samples were repeatedly frozen, thawed, refrozen. However, after 30 and 90 days of freezing, the increase was not significant. Specifically, after three cycles of freezing and thawing, MDA levels in the chicken breast samples increased from 0.253 to 0.646 nmol/mg, indicating an increase in lipid oxidation. Additionally, during the refreezing period, there was a notable (P≤0.05) increase in MDA concentrations as the storage time increased, from 0.253 nmol/mg to 2.136 nmol/mg. This increase may be attributed to the production of pro-oxidants, such as heme pigments, degraded lipids, and free radicals, along with oxidative enzymes, including lipases, nucleases, and proteases. These substances often come from damaged cellular organelles, and freeze-thaw cycles appear to accelerate lipid oxidation (Sun et al., 2019). Wu et al. (2021) found that fat in raw meat continues to oxidize even after multiple freeze-thaw cycles, leading to an increase in malondialdehyde levels. Specifically, one freeze-thaw cycle increased the (MDA) level in fresh meat, while seven cycles produced the highest concentration. Our results align with those of Ali et al. (2015) regarding chicken meat and Pan et al. (2021) concerning pork patties, both of which also indicated a connection between lipid oxidation and the number of freeze-thaw cycles.

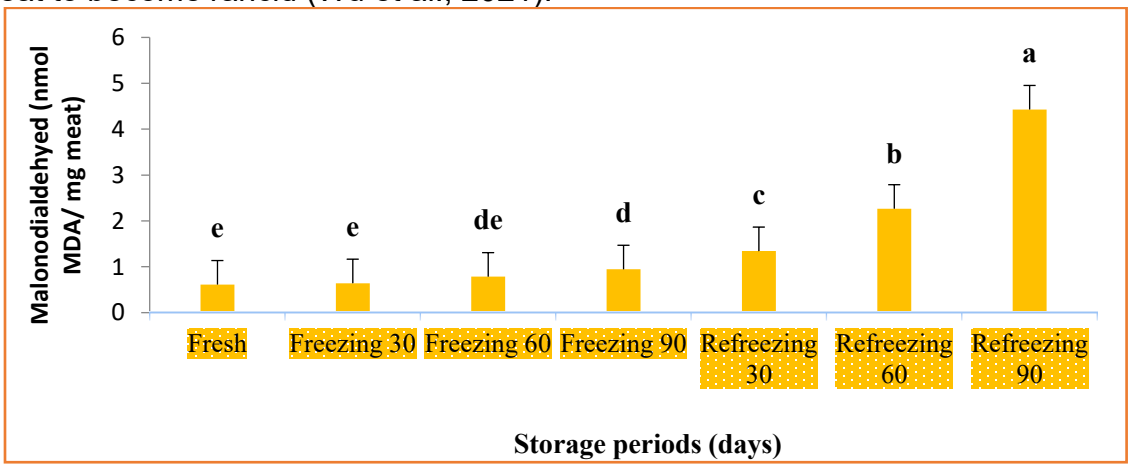

Figure1. The effect of freeze-thaw-refreeze cycles on lipid oxidation of broiler chicken breast meat

[a-e]The variations are indicated by distinct letters located in the same row (P < 0.05)

3.5 Sulfhydryl Content (Thiol Content) in broiler chicken breast meat

After excluding the 30-day freezing period, content of thiol in samples of broiler meat generally decreased subsequent to multiple freeze-thaw-refreeze cycles. Specifically, the levels of sulfhydryl groups decreased significantly (P≤0.05) from 65.243 to 33.960 after three cycles of freezing and thawing (Figure 2). It has been observed that the oxidation of sulfhydryl groups leads to various byproducts, including sulfinic acid and disulfide cross-links. Initially, the thiol concentration in the breast meat was 65.243 nmol mg-1 protein. Over the storage period, the sulfhydryl groups gradually diminished, with a particularly notable decline during refreezing for 90 days (P≤0.05). The thiol contents in breast meat samples were measured at range approximately between (56.123-33.960 nmol/mg protein) (P≤0.05) after 90 days of freezing refreezing, respectively. The reduction in thiol levels may be linked to denaturation and coagulation of muscle proteins, which can occur during storage. Specifically, the process of oxidizing thiol groups in cysteine or swapping disulfide bonds among polypeptides can occur (Xia et al., 2009).

Supporting our results, a study on turkey breast meat stored for three weeks at -30°C also showed a significant decline in relation to both reactive thiol groups and total thiol groups (Chan et al., 2011). Additionally, Soyer et al. (2010) indicated that the duration of freezing can greatly impact protein oxidation, as evidenced by a reduction in sulfhydryl levels over six months of frozen storage. Heme iron is crucial for initiating protein carbonylation in meat. Certain amino acids, such as threonine, lysine, proline, and arginine, are particularly vulnerable to oxidation, which is facilitated by the heme iron (Lund et al., 2011; Utrera and Estevez, 2013). Additionally, these findings align with Soncu's (2020) research, which reported a significant decrease (P≤0.05) in levelof thiol in both breast and thigh samples after storage for six (6) months in freezer. Moreover, Rinwi et al. (2023) observed a significant decrease (P<0.05) in the amount of sulfhydryl in chicken breast samples frozen at -8 and -12 °C. The thiol-level was reduced insignificantly during frozen storage at -4 °C in comparison with the control samples (untreated samples). According to previous studies, the formation of disulfide bonds, either within or across polypeptide chains, may cause thiol loss in meat after freezing (Soncu, 2020)

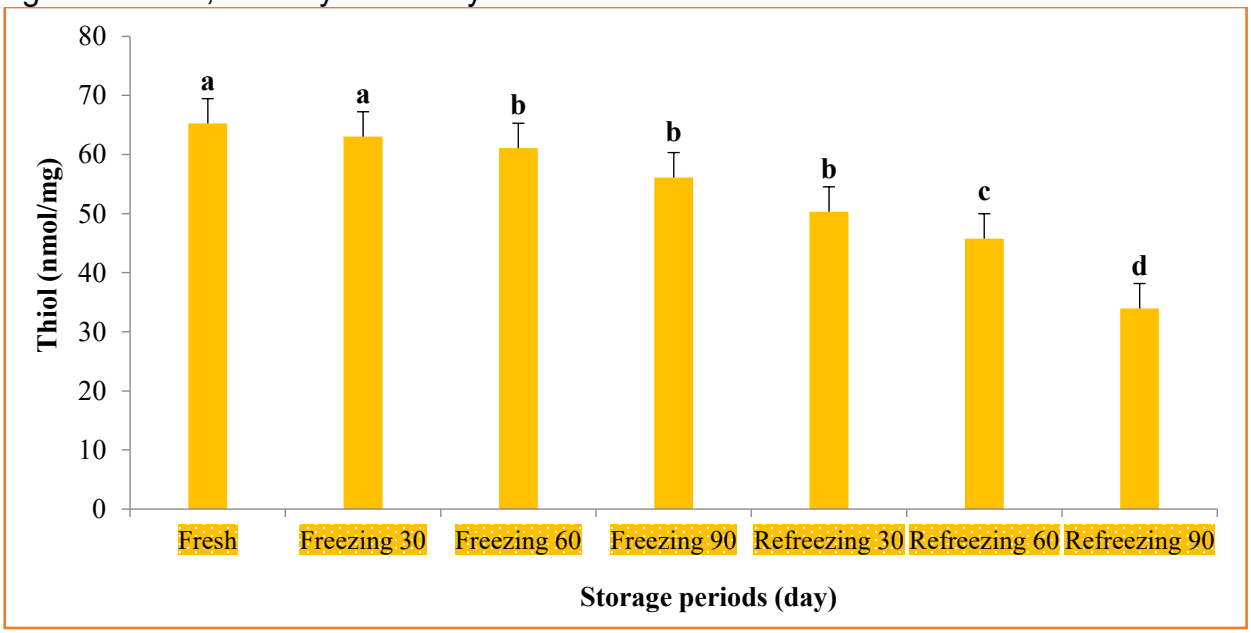

Figure2. The effect of freeze- thaw-refreeze cycles on thiol content of broiler chicken breast meat

[a-d]The variations are indicated by distinct letters located in the same row (P < 0.05)

3.6 Carbonyl content in broiler chicken breast meat

Carbonyl group production is a well-known process that explains the chemical changes in amino acids and is commonly used as an indicator of protein oxidation (Zou *et al*., 2018). **Figure 3** illustrates the changes in carbonyl concentrations in broiler-breast samples during freezing and refreezing. Notably, after several cycles of freezing, thawing, and refreezing, the carbonyl levels increased significantly (P≤0.05), with the exception of the 30-day freezing period, where the increase was not significant. The control group, which did not undergo freezing, showed the lowest carbonyl level (17.966 nmol/mg protein). In contrast, after 90 days of repeated freeze/thaw cycles of freezing and thawing, the carbonyl level peaked at 39.720 nmol/mg protein. This indicated that the frequency of freeze-thaw-refreeze cycles contributed to increased protein oxidation in breast broiler samples. Our results are consistent with those of previous studies, including those by Pan *et al*. (2021) on pork, Chan *et al*. (2011) on turkey, Lund *et al*. (2011) on fish, Utrera and Estevez (2013) on chicken, and Utrera *et al*. (2014) on beef patties, all of which reported an increase in carbonyl content following freezing. Additionally, the results align with those of Soncu (2020), who found a significant increase in carbonyl content (P≤0.05) in chicken breast and thigh after six months of freezing. Furthermore, our results are in line with the findings of Rinwi*et al*. (2023), who indicated that the amount of carbonyl increased considerably (P<0.05) in breast samples during freezing storage at -8 and -12 °C. Rinwi *et al*. (2023) explained that the increases in carbonyl levels at low temperatures might be attributed to chemical changes in amino acids, such as the release of oxidizing enzymes and pro-oxidant compounds from damaged organelles in cells (Soncu, 2020 and Zou *et al*., 2018). During storage at freezing and refreezing temperatures, various factors including hydroxyl radicals contribute to the oxidation of proteins in the chicken breast samples (Silva *et al*., 2018).

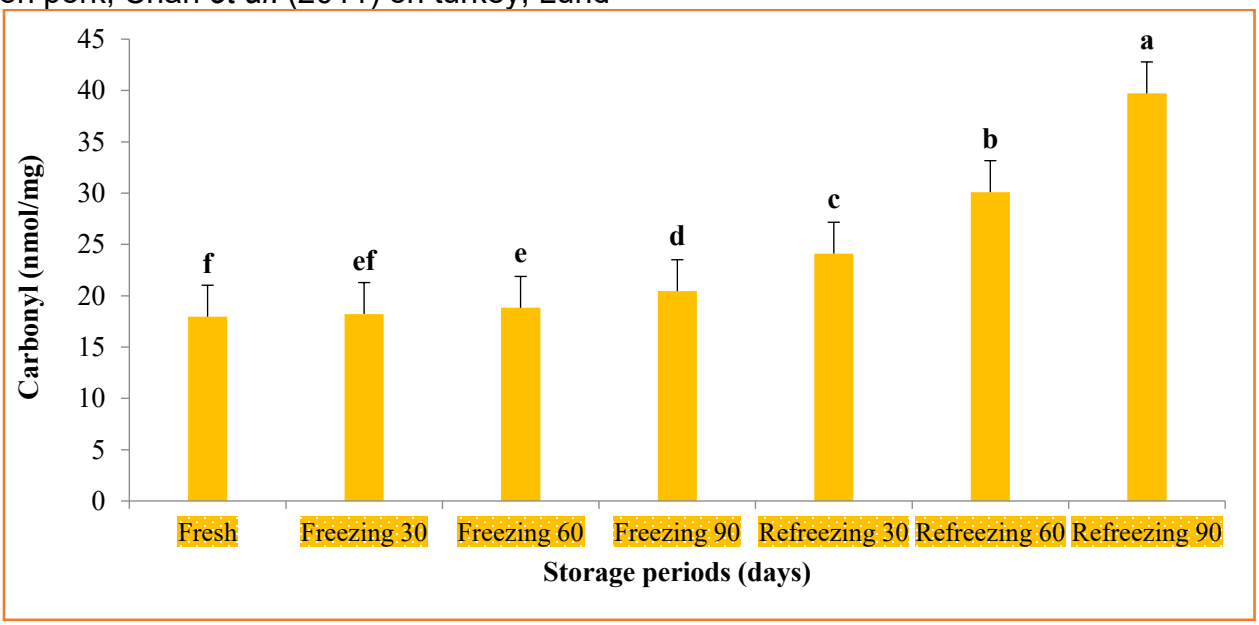

Figure 3. The effect of freeze-thaw-refreeze cycleson carbonyl content of broilerchicken breastmeat
[a-f]The variations are indicated by distinct letters located in the same row (P < 0.05)

3.7 Myoglobin content in broiler chicken breast meat

Myoglobin is a protein found in muscle tissue that gives meat a characteristic red color. This protein consists of a polypeptide chain shaped like an alpha helix and contains an iron atom at its center, known as the heme group. The color of meat primarily depends on the structure of

myoglobin and the amount of this pigment. Factors such as the level iron oxidation and the presence of compounds for instance availability of oxygen, water in free forms, and nitric oxide (NO) play crucial roles in identifying the ultimate hue (color) of meat (Coria et al., 2020). The oxidation of bright oxymyoglobin and purple deoxy myoglobin can lead to the formation of brown metmyoglobin on the surface of meat, which results in a change in color. Myoglobin, along with its associated ligands, undergoes reduction and oxidation processes that give the meat its color. Several factors can affect the color of meat, including both internal and external factors. Meat color is influenced by multiple factors, which can be categorized as internal or external. These factors include pH, oxygen availability, oxidation of lipid, aging after death(postmortem aging), variations in temperature, freezing methods, and casing (packaging) utilized (Mancini and Hunt, 2005; Suman and Joseph, 2013). The findings from this study indicate that the myoglobin content in broiler breast samples decreased only slightly with increasing the storage duration freezing and thawing (see Figure 4). It is evident that prolonged freezing times result in heightened myoglobin oxidation throughout multiple freeze-thaw cycles. However, this oxidation occurs at a slower rate in breast samples compared to those that have been refrozen samples.

As the duration of refrozen storage increased, the myoglobin levels in the meat significantly decreased ($P \leq 0.05$). This decline occurs because physical damage from ice crystals disrupts cells and their internal structures, leading to a greater exposure of lipids and myoglobin to oxidation-promoting chemicals. A direct link excites between lipid oxidation and myoglobin, as noted by Mancini and Hunt (2005). Moreover, the processes of freezing, thawing, and refreezing meat increase the likelihood of interactions with pro-oxidative substances. These cycles can also denature the myoglobin molecule, elevating the solute concentration inside the cellular structure, and eventually leading to the myoglobin oxidation (Aroeira et al., 2017). When meat thaws, the globin portion of myoglobin becomes denatured, making it more prone to auto-oxidation, which explains the rapid discoloration observed in thawed meat, as highlighted by (Abdulla et al., 2019). These observations are consistent with the findings of Wang et al. (2018) and Aroeira et al. (2017), who reported a decrease in myoglobin levels after several freeze-thaw cycles.

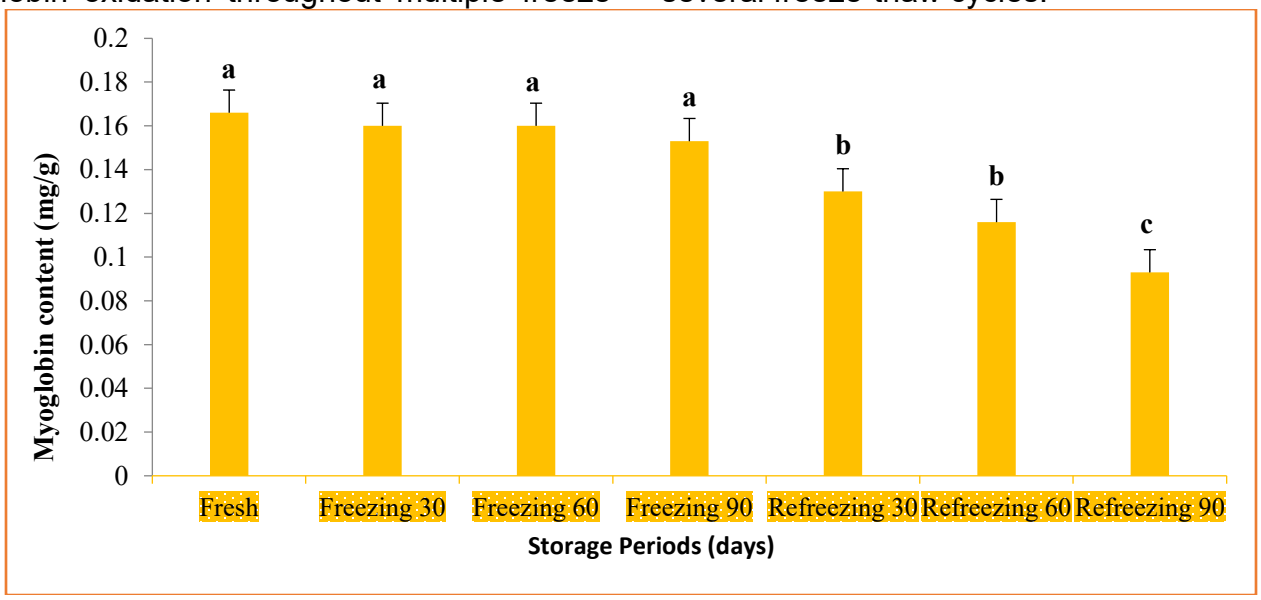

Figure 4: The effect of freeze-thaw-refreeze cycles on myoglobin content of broiler chicken breast meat

[a-f]The variations are indicated by distinct letters located in the same row ($P < 0.05$)

Conclusion

The study highlights the adverse impact of freeze-thaw-refreeze cycles on the nutritional and compositional quality of broiler breast meat. Prolonged freezing and refreezing storage significantly reduced the levels of essential and non-essential amino acids and fatty acids, with oxidative changes causing further degradation. Such essential nutritional components as unsaturated fatty acids and essential amino acids like leucine, lysine, and valine were most affected, with remarkable decreases obtained with increased storage duration. Refreezing also enhanced lipid and protein oxidation, which played a part in remarkable alterations of the nutritional quality of the samples. The findings emphasize the importance of minimizing repeated freezing and refreezing to preserve the essential nutritional value of meat products.

References

Abdulla, N.R., Loh, T.C., Foo, H.L., Alshelmani, M.I., Akit, H., (2019). Influence of dietary ratios of n-6: n-3 fatty acid on gene expression, fatty acid profile in liver and breast muscle tissues, serum lipid profile, and immunoglobulin in broiler chickens. *Journal of Applied Poultry Research* 28, 454–469.

Abdullahi Bida, A., (2019). Storage and Cooling Effects on Levels of Protein Carbohydrates, Fats, Minerals, and Microbes in Guinea Fowl (Numida meleagris) Meat Sold in Kaduna Metropolis, Nigeria. *Advances in Biochemistry* 7, 10.

Abubakar, A.A., Zulkifli, I., Goh, Y.M., Kaka, U., Sabow, A.B., Imlan, J.C., Awad, E.A., Othman, A.H., Raghazli, R., Mitin, H., Sazili, A.Q., (2021). Effects of stocking and transport conditions on physicochemical properties of meat and acute-phase proteins in cattle. *Foods* 10, 252.

Adedokun, S.A., Jaynes, P., Abd El-Hack, M.E., Payne, R.L., Applegate, T.J., (2014). Standardized ileal amino acid digestibility of meat and bone meal and soybean meal in laying hens and broilers. *Poultry Science* 93, 420–428.

Ahmed, B.H., Marzany, N.A., (2022). Microbial counts and eating quality of breast broilers meat subjected to different freezing and refreezing storage periods. *Tikrit Journal for Agricultural Sciences* 22, 53–59.

Akinnusi, F.A.O., Oni, O.O., Ademolu, K.O., (2018). Mineral composition of giant African land snail's (Archachatina marginata) shells from six South West States, Nigeria. *Nigerian Journal of Animal Science* 20, 485–489.

Aladdin, D., Ismail, A. H., Taha, A. H., & Hussein, Z. A. (2022). Evaluation of the trace element concentration in some livestock and poultry bone samples using X-ray fluorescence. *Zanco Journal of Pure and Applied Sciences* 34(4), 67–73.

Ali, S., Zhang, W., Rajput, N., Khan, M.A., Li, C.B., Zhou, G.H., (2015). Effect of multiple freeze–thaw cycles on the quality of chicken breast meat. *Food Chemistry* 173, 808–814.

Alina, H., Ovidiu, T., (2007). Determination of total protein in some meat products. Analele Stiintifice ale Universitatii Alexandru Ioan Cuza Sectiunea Genetica si Biologie Moleculara 8.

Aroeira C.N., Torres Filho, R.A., Fontes, P.R., Ramos, A.L., Gomide, L.A., Ladeira, M.M., Ramos, E.M., (2017). Effect of freezing prior to aging on myoglobin redox forms and CIE color of beef from Nellore and Aberdeen Angus cattle. *Meat Science* 125, 16–21.

Biesalski, H.K., (2005). Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet? *Meat Science* 70, 509–524.

Carvalho, R.H., Soares, A.L., Grespan, M., Spurio, R.S., Coró, F.A.G., Oba, A., Shimokomaki, M., (2015). The effects of the dark house system on growth, performance and meat quality of broiler chicken. *Animal Science Journal* 86, 189–193.

Chan, J.T.Y., Omana, D.A., Betti, M., (2011). Effect of ultimate pH and freezing on the biochemical properties of proteins in turkey breast meat. *Food Chemistry* 127, 109–117.

Charoenrein, S., (2018). Microstructural changes and their relationship with quality and stability of frozen foods. *Food Microstructure and Its Relationship with Quality and Stability*, 123–138.

Chen, Q., (2024). Protein Structure Prediction. Association Analysis Techniques and Applications in Bioinformatics 205–235.

Coombs, C.E.O., Holman, B.W.B., Friend, M.A., Hopkins, D.L., (2017). Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Science* 125, 84–94.

Coria-Hernández, J., Meléndez-Pérez, R., Méndez-Albores, A., Arjona-Román, J.L., (2020). Changes in myoglobin content in pork Longissimus thoracis muscle during freezing storage. *Revista Mexicana de Ciencias Pecuarias* 11, 651–668.

Farhan, S.M., Alnori, H.M., Yousif, S.I., Mirzan, N.A., Abdulateef, S.M., Jebril, N., Al-Azzami, A.A., Saeed, O.A., Sulaiman, B.F., Sardary, S.Y.T., (2024). Innovative strategies in the use of Vitamin E and rosemary as antimicrobial agents for enhancing the safety and preservation of minced poultry meat during refrigeration. *Anbar Journal of Agricultural Sciences* 22, 1570–1580.

Hammad, H., Ma, M., Hydamaka, A.W., Elkhedir, A.E., Jin, G., (2019). Effect of Freeze and Re-freeze on Chemical Composition of Beef and Poultry Meat at Storage Period 4.5 Months (SP4.5). *Journal of Food Processing and Technology* 10, 791.

Hassan Ahmed, B., Abdulqadir Marzany, N., (2022). Physical and chemical properties of breast chicken meat subjected to different freezing and refreezing storage periods. International *Journal of Special Education* 37, 17539–17549.

Hou, Y., Yin, Y., Wu, G., (2015). Dietary essentiality of "nutritionally non-essential amino acids" for animals and humans. *Experimental Biology and Medicine* 240, 997.

Kaczmarek, A., Muzolf-Panek, M., (2021). Prediction of Thiol Group Changes in Minced Raw and Cooked Chicken Meat with Plant Extracts—Kinetic and Neural Network Approaches. *Animals* 11, 1647.

Leygonie, C., Britz, T.J., Hoffman, L.C., (2012). Impact of freezing and thawing on the quality of meat: A review. *Meat Science* 91, 93–98.

Lund, M.N., Heinonen, M., Baron, C.P., Estévez, M., (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research* 55, 83–95.

Macelline, S.P., Chrystal, P.V., Liu, S.Y., Selle, P.H., (2021). The dynamic conversion of dietary protein and amino acids into chicken-meat protein. *Animals* 11, 2288.

Majewska, D., Jakubowska, M., Ligocki, M., Tarasewicz, Z., Szczerbińska, D., Karamucki, T., Sales, J., (2009). Physicochemical characteristics, proximate analysis, and mineral composition of ostrich meat as influenced by muscle. *Food Chemistry* 117, 207–211.

Mancini, R.A., Hunt, M.C., (2005). Current research in meat color. *Meat Science* 71, 100–121.

Mititelu, M., Lupuliasa, D., Neacșu, S.M., Olteanu, G., Busnatu, Ș.S., Mihai, A., Popovici, V., Măru, N., Boroghină, S.C., Mihai, S., Ioniță-Mîndrican, C.B., Scafa-Udriște, A., (2024). Polyunsaturated Fatty Acids and Human Health: A Key to Modern Nutritional Balance in Association with Polyphenolic Compounds from Food Sources. *Foods* 14, 46.

Mohammed, H.H.H., He, L., Nawaz, A., Jin, G., Huang, X., Ma, M., Abdegadir, W.S., Elgasim, E.A., Khalifa, I., (2021). Effect of frozen and refrozen storage of beef and chicken meats on inoculated microorganisms and meat quality. *Meat Science* 175.

Pan, N., Dong, C., Du, X., Kong, B., Sun, J., Xia, X., (2021). Effect of freeze-thaw cycles on the quality of quick-frozen pork patty with different fat content by consumer assessment and instrument-based detection. *Meat Science* 172, 108313.

Park, S.Y., Yoo, S.S., Uh, J.H., Eun, J.B., Lee, H.C., Kim, Y.J., Chin, K.B., (2007). Evaluation of lipid oxidation and oxidative products as affected by pork meat cut, packaging method, and storage time during frozen storage (-10°C). Journal of *Food Science* 72.

Rahman, M., Hashem, M., Azad, M., Choudhury, M., Bhuiyan, M., (2023). Techniques of meat preservation – A review. *Meat Research* 3, 1971.

Rinwi, T.G., Sun, D.W., Ma, J., Wang, Q.J., (2023). Effects of isochoric freezing on freezing process and quality attributes of chicken breast meat. *Food Chemistry* 405, 134732.

Saber, T.D., (2023). Effect of sheep and chicken manure on wheat growth and productivity. *Zanco Journal of Pure and Applied Sciences* 35(6), 113–117.

Sabagh, E., Taha, N., Saleh, E., ElFar, A., Sadek, K., (2016). Effect of Freezing and Frozen Storage on Amino Acid Profile and Fatty Acid Pattern in Imported and Local Meat. *Alexandria Journal of Veterinary Sciences* 49, 113–121.

SAS Institute, (2001). Statistical Analysis System User's Guide: Statistics. SAS Institute, NC, USA.

Silva, F.A.P., Estévez, M., Ferreira, V.C.S., Silva, S.A., Lemos, L.T.M., Ida, E.I., Shimokomaki, M., Madruga, M.S., (2018). Protein and lipid oxidations in jerky chicken and consequences on sensory quality. LWT 97, 341–348.

Soncu, E.D., (2020). Protein oxidation and subsequent changes in chicken breast and thigh meats during long-term frozen storage. Agricultural and Food Science 29, 505–514.

Soyer, A., Özalp, B., Dalmiş, Ü., Bilgin, V., (2010). Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food Chemistry* 120, 1025–1030.

Suman, S.P., Joseph, P., (2013). Myoglobin chemistry and meat color. Annual Review of *Food Science and Technology* 4, 79–99.

Sun, Q., Chen, Q., Xia, X., Kong, B., Diao, X., (2019). Effects of ultrasound-assisted freezing at different power levels on the structure and thermal stability of common carp (Cyprinus carpio) proteins. *Ultrasonics Sonochemistry* 54, 311–320.

Utrera, M., Estévez, M., (2013). Oxidative damage to poultry, pork, and beef during frozen storage through the analysis of novel protein oxidation markers. *Journal of Agricultural and Food Chemistry* 61, 7987–7993.

Utrera, M., Morcuende, D., Estévez, M., (2014). Temperature of frozen storage affects the nature and consequences of protein oxidation in beef patties. *Meat Science* 96, 1250–1257.

Viveros, A., Brenes, A., Arija, I., Centeno, C., (2002). Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. *Poultry Science* 81, 1172–1183.

Abdulla, N.R., Loh, T.C., Foo, H.L., Alshelmani, M.I., Akit, H., (2019). Influence of dietary ratios of n-6: n-3 fatty acid on gene expression, fatty acid profile in liver and breast muscle tissues, serum lipid profile, and immunoglobulin in broiler chickens. *Journal of Applied Poultry Research* 28, 454–469.

Abdullahi Bida, A., (2019). Storage and Cooling Effects on Levels of Protein Carbohydrates, Fats, Minerals, and Microbes in Guinea Fowl (Numida meleagris) Meat Sold in Kaduna Metropolis, Nigeria. *Advances in Biochemistry* 7, 10.

Abubakar, A.A., Zulkifli, I., Goh, Y.M., Kaka, U., Sabow, A.B., Imlan, J.C., Awad, E.A., Othman, A.H., Raghazli, R., Mitin, H., Sazili, A.Q., (2021). Effects of stocking and transport conditions on physicochemical properties of meat and acute-phase proteins in cattle. *Foods* 10, 252. .

Adedokun, S.A., Jaynes, P., Abd El-Hack, M.E., Payne, R.L., Applegate, T.J., (2014). Standardized ileal amino acid digestibility of meat and bone meal and soybean meal in laying hens and broilers. *Poultry Science* 93, 420–428.

Ahmed, B.H., Marzany, N.A., (2022). Microbial counts and eating quality of breast broilers meat subjected to different freezing and refreezing storage periods. *Tikrit Journal for Agricultural Sciences* 22, 53–59.

Akinnusi, F.A.O., Oni, O.O., Ademolu, K.O., (2018). Mineral composition of giant African land snail's (Archachatina marginata) shells from six South West States, Nigeria. *Nigerian Journal of Animal Science* 20, 485–489.

Aladdin, D., Ismail, A. H., Taha, A. H., & Hussein, Z. A. (2022). Evaluation of the trace element concentration in some livestock and poultry bone samples using X-ray fluorescence. *Zanco Journal of Pure and Applied Sciences* 34(4), 67–73.

Ali, S., Zhang, W., Rajput, N., Khan, M.A., Li, C.B., Zhou, G.H., (2015). Effect of multiple freeze–thaw cycles on the quality of chicken breast meat. *Food Chemistry* 173, 808–814.

Alina, H., Ovidiu, T., (2007). Determination of total protein in some meat products. Analele Stiintifice ale Universitatii Alexandru Ioan Cuza Sectiunea Genetica si Biologie Moleculara 8.

Aroeira, C.N., Torres Filho, R.A., Fontes, P.R., Ramos, A.L., Gomide, L.A., Ladeira, M.M., Ramos, E.M., (2017). Effect of freezing prior to aging on myoglobin redox forms and CIE color of beef from Nellore and Aberdeen Angus cattle. *Meat Science* 125, 16–21.

Biesalski, H.K., (2005). Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet? *Meat Science* 70, 509–524.

Carvalho, R.H., Soares, A.L., Grespan, M., Spurio, R.S., Coró, F.A.G., Oba, A., Shimokomaki, M., (2015). The effects of the dark house system on growth, performance and meat quality of broiler chicken. *Animal Science Journal* 86, 189–193.

Chan, J.T.Y., Omana, D.A., Betti, M., (2011). Effect of ultimate pH and freezing on the biochemical properties of proteins in turkey breast meat. *Food Chemistry* 127, 109–117.

Charoenrein, S., (2018). Microstructural changes and their relationship with quality and stability of frozen foods. *Food Microstructure and Its Relationship with Quality and Stability*, 123–138.

Chen, Q., (2024). Protein Structure Prediction. Association Analysis Techniques and Applications in Bioinformatics 205–235.

Coombs, C.E.O., Holman, B.W.B., Friend, M.A., Hopkins, D.L., (2017). Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Science* 125, 84–94.

Coria-Hernández, J., Meléndez-Pérez, R., Méndez-Albores, A., Arjona-Román, J.L., (2020). Changes in myoglobin content in pork Longissimus thoracis muscle during freezing storage. *Revista Mexicana de Ciencias Pecuarias* 11, 651–668.

Farhan, S.M., Alnori, H.M., Yousif, S.I., Mirzan, N.A., Abdulateef, S.M., Jebril, N., Al-Azzami, A.A., Saeed, O.A., Sulaiman, B.F., Sardary, S.Y.T., (2024). Innovative strategies in the use of Vitamin E and rosemary as antimicrobial agents for enhancing the safety and preservation of minced poultry meat during refrigeration. *Anbar Journal of Agricultural Sciences* 22, 1570–1580.

Hammad, H., Ma, M., Hydamaka, A.W., Elkhedir, A.E., Jin, G., (2019). Effect of Freeze and Re-freeze on Chemical Composition of Beef and Poultry Meat at Storage Period 4.5 Months (SP4.5). *Journal of Food Processing and Technology* 10, 791.

Hassan Ahmed, B., Abdulqadir Marzany, N., (2022). Physical and chemical properties of breast chicken meat subjected to different freezing and refreezing storage periods. International *Journal of Special Education* 37, 17539–17549.

Hou, Y., Yin, Y., Wu, G., (2015). Dietary essentiality of “nutritionally non-essential amino acids” for animals and humans. *Experimental Biology and Medicine* 240, 997.

Kaczmarek, A., Muzolf-Panek, M., (2021). Prediction of Thiol Group Changes in Minced Raw and Cooked Chicken Meat with Plant Extracts—Kinetic and Neural Network Approaches. *Animals* 11, 1647.

Leygonie, C., Britz, T.J., Hoffman, L.C., (2012). Impact of freezing and thawing on the quality of meat: A review. *Meat Science* 91, 93–98.

Lund, M.N., Heinonen, M., Baron, C.P., Estévez, M., (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research* 55, 83–95.

Macelline, S.P., Chrystal, P.V., Liu, S.Y., Selle, P.H., (2021). The dynamic conversion of dietary protein and amino acids into chicken-meat protein. *Animals* 11, 2288.

Majewska, D., Jakubowska, M., Ligocki, M., Tarasewicz, Z., Szczerbińska, D., Karamucki, T., Sales, J., (2009). Physicochemical characteristics, proximate analysis, and mineral composition of ostrich meat as influenced by muscle. *Food Chemistry* 117, 207–211.

Mancini, R.A., Hunt, M.C., (2005). Current research in meat color. *Meat Science* 71, 100–121.

Mititelu, M., Lupuliasa, D., Neacșu, S.M., Olteanu, G., Busnatu, Ș.S., Mihai, A., Popovici, V., Măru, N., Boroghină, S.C., Mihai, S., Ioniță-Mîndrican, C.B., Scafa-Udriște, A., (2024). Polyunsaturated Fatty Acids and Human Health: A Key to Modern Nutritional Balance in Association with Polyphenolic Compounds from Food Sources. *Foods* 14, 46.

Mohammed, H.H.H., He, L., Nawaz, A., Jin, G., Huang, X., Ma, M., Abdegadir, W.S., Elgasim, E.A., Khalifa, I., (2021). Effect of frozen and refrozen storage of beef and chicken meats on inoculated microorganisms and meat quality. *Meat Science* 175.

Pan, N., Dong, C., Du, X., Kong, B., Sun, J., Xia, X., (2021). Effect of freeze-thaw cycles on the quality of quick-frozen pork patty with different fat content by consumer assessment and instrument-based detection. *Meat Science* 172, 108313.

Park, S.Y., Yoo, S.S., Uh, J.H., Eun, J.B., Lee, H.C., Kim, Y.J., Chin, K.B., (2007). Evaluation of lipid oxidation and oxidative products as affected by pork meat cut, packaging method, and storage time during frozen storage (-10°C). Journal of *Food Science* 72.

Rahman, M., Hashem, M., Azad, M., Choudhury, M., Bhuiyan, M., (2023). Techniques of meat preservation – A review. *Meat Research* 3, 1971.

Rinwi, T.G., Sun, D.W., Ma, J., Wang, Q.J., (2023). Effects of isochoric freezing on freezing process and quality attributes of chicken breast meat. *Food Chemistry* 405, 134732.

Saber, T.D., (2023). Effect of sheep and chicken manure on wheat growth and productivity. *Zanco Journal of Pure and Applied Sciences* 35(6), 113–117.

Sabagh, E., Taha, N., Saleh, E., ElFar, A., Sadek, K., (2016). Effect of Freezing and Frozen Storage on Amino Acid Profile and Fatty Acid Pattern in Imported and Local Meat. *Alexandria Journal of Veterinary Sciences* 49, 113–121.

SAS Institute, (2001). Statistical Analysis System User's Guide: Statistics. SAS Institute, NC, USA.

Silva, F.A.P., Estévez, M., Ferreira, V.C.S., Silva, S.A., Lemos, L.T.M., Ida, E.I., Shimokomaki, M., Madruga, M.S., (2018). Protein and lipid oxidations in jerky chicken and consequences on sensory quality. LWT 97, 341–348.

Soncu, E.D., (2020). Protein oxidation and subsequent changes in chicken breast and thigh meats during long-term frozen storage. Agricultural and Food Science 29, 505–514.

Soyer, A., Özalp, B., Dalmiş, Ü., Bilgin, V., (2010). Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food Chemistry* 120, 1025–1030.

Suman, S.P., Joseph, P., (2013). Myoglobin chemistry and meat color. Annual Review of *Food Science and Technology* 4, 79–99.

Sun, Q., Chen, Q., Xia, X., Kong, B., Diao, X., (2019). Effects of ultrasound-assisted freezing at different power levels on the structure and thermal stability of common carp (Cyprinus carpio) proteins. *Ultrasonics Sonochemistry* 54, 311–320.

Utrera, M., Estévez, M., (2013). Oxidative damage to poultry, pork, and beef during frozen storage through the analysis of novel protein oxidation markers. *Journal of Agricultural and Food Chemistry* 61, 7987–7993.

Utrera, M., Morcuende, D., Estévez, M., (2014). Temperature of frozen storage affects the nature and consequences of protein oxidation in beef patties. *Meat Science* 96, 1250–1257.

Viveros, A., Brenes, A., Arija, I., Centeno, C., (2002). Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. *Poultry Science* 81, 1172–1183.

Wang, Z., He, Z., Gan, X., Li, H., (2018). The effect of repeated freeze-thaw cycles on the meat quality of rabbit. *World Rabbit Science* 26, 165–177.

Wu, X., Zhang, Z., He, Z., Wang, Z., Qin, F., Zeng, M., Chen, J., (2021). Effect of freeze-thaw cycles on the oxidation of protein and fat and its relationship with the formation of heterocyclic aromatic amines and advanced glycation end products in raw meat. *Molecules* 26, 1264.

Xia, X., Kong, B., Liu, Q., Liu, J., (2009). Physicochemical change and protein oxidation in porcine Longissimus dorsi as influenced by different freeze-thaw cycles. *Meat Science* 83, 239–245.

Zhang, Y., Ertbjerg, P., (2019). On the origin of thaw loss: Relationship between freezing rate and protein denaturation. *Food Chemistry* 299, 125104.

Zou, Y., Xu, P., Wu, H., Zhang, M., Sun, Z., Sun, C., Wang, D., Cao, J., Xu, W., (2018). Effects of different ultrasound power on physicochemical property and functional performance of chicken actomyosin. *International Journal of Biological Macromolecules* 113, 640–647.