EFFECT OF GREEN WALNUT HULL (*Juglans regia L.)* EXTRACT ON OXIDATIVE STABILITY, COLOR CHARACTERISTICS, MICROBIAL QUALITY, AND LIPID INDICES OF KARADI LAMB MEAT

Arazu Abdullah Hama[1] and Lana Aram Kamal[2]

[1] Animal Science Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sualimani, Iraq.

[2] The International School of Choueifat, Sulaimani, Iraq.

arazw.hama@univsul.edu.iq lanaa.aramm08@gmail.com

Abstract

Background: Sheep meat is highly to deterioration throughout refrigerated storage due to rapid microbial growth and oxidative deterioration of lipids and muscle pigments, these changes can reduce shelf life. Green walnut hulls (*Juglans regia L*.) which are contain phenolic compounds, have attracted interest as a potential clean label source. **Aims:** To assess green walnut hull extract as a natural antioxidant, antimicrobial for Karadi lamb *semimembranosus*, with emphasis on oxidative stability, color preservation, microbial quality, fatty acid stability, and lipid health indices. **Methodology:** Green walnut hull extract was obtained by hydroethanolic (50% ethanol–water) ultrasound-assisted extraction and characterized for total phenolic content (Folin–Ciocalteu). Lamb *semimembranosus* slices were dipped for 24 h at 4 °C in distilled water (T1) or extract solutions at 1% (T2), 2% (T3), and 3% (T4), then kept at 4 °C for conduct analyses on days 1, 3, 6, and 9 for TBARS, CIE color parameters, microbial counts (TPC, psychrotrophs, coliforms), fatty acid profile (GC-FID), and lipid health indices. Data were evaluated by two way ANOVA and Duncan's multiple test **Results:** Extract TPC was 38.2 mg GAE/g DM. TBARS increased during storage ($p \leq 0.01$) but remained significantly lower in extract-treated meat; at day 9, TBARS was highest in T1 and lowest in T4. Color stability was improved by the extract; at day 9, L* and a* were lowest in T1 and highest in T4. Microbial counts increased with time but were consistently reduced by the extract; at day 9, TPC was higher in T1 while was lower in T4, with similar reductions in psychrotrophs and coliforms. Storage reduced PUFA; however, T4 showed higher PUFA retention at day 9 than T1 and better preservation of C18:3 n-3 and CLA. Lipid health indices also showed improvment in treated samples at day 9, with higher PUFA/SFA, lower n-6/n-3, and reduced AI and TI ($p \leq 0.01$). **Conclusion:** Green walnut hull extract, particularly at 3% (T4), significantly enhanced chilled lamb meat stability by lowering lipid oxidation, preserving colour, suppressing microbial growth, and maintaining a more favourable fatty-acid profile and lipid nutritional indices, supporting its use as a clean-label preservative.

Key words: green walnut hull; lamb meat; meat shelf life; oxidation; color stability; microbial counts; fatty acids; health indices.

Introduction

Lamb meat is highly perishable, usually maintains acceptable quality for about 7–10

ISSN 2072-3857

days under refrigeration condition storage [34]. This limited shelf life is primarily as aresult of its high water activity and near neutral pH, which create favorable condition of rapid microbial growth and spoilage [29]. In addition to microbial deterioration, lamb meat is vulnerable to oxidative deterioration; lipid oxidation generates aldehydes and ketones that cause off odors, rancidity, and sensory unacceptable [21] . Collectively, these deterioration process contribute to product rejection and food waste, posing economic burdens on the meat industry [17]. Therefore, effective preservation strategies require to maintain meat quality during chilled storage.

Synthetic antioxidants sources have been widely used in fresh meat in order to delay lipid oxidation; however, evidence of potential human health risks has led to stricter regulations and growing consumer resistance and increased preference for natural alternatives [14]. Consequently, the food industry and scientific community are increasingly turning to natural, plant sources with similar preservation effects avoiding health concerns [27]. Recent research highlighted the effectiveness of antioxidants obtained from herbs, spices, fruits, and other botanicals in preventing spoilage and improve meat stability, while also providing anti inflammatory and clean label benefits [13].

Many plants derived compounds exhibit dual functionality antioxidant and antimicrobial allowing them to suppress the oxidation of lipid and protein as well as limit the proliferation of spoilage microorganisms [13]. Lamb meat contains a considerable proportion of unsaturated fatty acids, which is especially sensitive to oxidative rancidity, plant based as natural antioxidants have proven effective in lamb and other meats reflects growing industry and consumer preferences for effective, natural preservative strategies [27, 35].

Green walnut hulls (*Juglans regia L.*) are recognized as a valuable agro industrial by product, owing to their rich abundance of antioxidant and antimicrobial phytochemicals including polyphenolic constituents such as phenolic acids, flavonoids, and tocopherols, may underlie to their strong radical scavenging capacity [7,19]. Hydroethanolic or membrane assisted extraction methods enables the recovery of these bio actives in extracts with high total phenolic content and potent antioxidant capacity [6].

Furthermore, green walnut husk extracts have been showed significant in inhibiting growth of both Gram positive bacteria (e.g., *Staphylococcus aureus, Bacillus subtilis*) and Gram negative bacteria (e.g., *Escherichia coli*), which associated with their phenolic composition, compounds like juglone and ellagic acid [6,7]. These dual antioxidant and antimicrobial functionalities suggest that using green walnut hull extract not only aligns with sustainable waste utilization practices but may serve as natural preservative suitable for food and cosmetic applications, increasing the shelf life of foods. [19].

Regardless of the well documented bioactivity of walnut husk extracts, their application in preserving fresh meat remains insufficiently studied. Most studies on walnut focused on its phytochemistry or use in pharmaceutical and nutraceutical uses, with few examining its efficacy in food preservation processes. Therefore, the present work was undertaken to determine the effectiveness of green walnut hull extract as a natural preservative for lamb meat during refrigeration storage. investigate whether it inhibit oxidative improvement

ISSN 2072-3857

and microbial growth, thereby prolonging shelf life and maintaining meat quality. This work contributes to the development of plant derived preservation strategies can achieve comparable protective effects to conventional additives, while meeting consumer demand for natural food ingredients.

Materials and Methods

Preparation of Green Walnut Hull Extract

Green walnut hull extract was prepared following the optimized hydroethanolic extraction protocol used by Savic & Savic Gajic [31]. Fresh green walnut hulls were separated, washed, oven-dried (40 °C) until constant weight. Then milled in to a fine powder by the use of a laboratory mill passed through a 1-mm sieve.

The hull powder was extracted with 50% ethanol water solvent (v/v) at a solvent to solid ratio of 10 mL/g. Then the mixture subjected to ultrasound-assisted extraction (UAE) in an ultrasonic bath operating at 40 kHz, to release of phenolic compounds. The extraction took place at room temperature for the optimized duration [31].

Following extraction, the mixture was filtered through Whatman No. 1 filter paper, and the solvent was subsequently removed using a rotary evaporator at 40 °C to obtain a semi solid crude extract, then freeze dried, and the resulting powder kept at 4 °C in airtight bags until use.

This extraction method was adopted from previously optimized protocol considered highly efficient which enhance the recovery of bioactive and other antioxidant constituents from green walnut hulls.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC), of the extract, was calculated using the Folin–Ciocalteu colorimetric assay in accordance with the procedure described by [12]. An aliquot extract was homogenized with diluted Folin Ciocalteu reagent, with addition of sodium carbonate (Na_2CO_3) solution, then allowed to stand in the absence of light, at room temperature for color development, by using UV–Vis spectrophotometer, absorbance measured was red at 765 nm.

Folin–Ciocalteu method reflects the extract's total reducing capacity, as the reagent reacts with both phenolic and non phenolic reducing molecules. TPC was calculated using a gallic acid calibration curve and expressed as mg gallic acid equivalents per gram of extract (mg GAE/g) as the following:

TPC (mg GAE/g) $= C \times V / m$
Where:
C = concentration obtained from the calibration curve (mg GAE/mL)
V = volume of extract used in the reaction (mL)
m = mass of extract in the tested solution (g)

Application of Green Walnut Hull Extract to Meat

Fresh *semimembranosus* muscles were collected from lambs (6–8-month-olds, 30 kg weight), after trimming visible fat and connective tissue, the muscles were cut to uniform slices (approximately 2 cm thick). The dried green walnut hull extract was reconstituted in distilled water to obtain different concentrations dipping solutions: 1%, 2%, and 3% (w/v). Control samples were immersed in distilled water only.

Each meat slice was immersed in 200 mL of the corresponding extract solution in tightly

ISSN 2072-3857

sealed plastic containers. and kept at 4 °C for the duration of 24 hours. Following immersion, the meat slices were put out from the solution and drained for 5 minutes on sterile racks to eliminate excess extract. All samples were packed in oxygen permeable polyethylene bags and kept at 4 °C, Meat analyses were conducted on Day 1, 3, 6, and 9 of storage.

Lipid Oxidation Measurement (TBARS)

The Meat lipid oxidation was analyzed using the thiobarbituric acid reactive substances (TBARS) assay, and were expressed as malondialdehyde (MDA). 10 g of meat samples were homogenized with trichloroacetic acid (TCA) solution (10%, w/v), filtered, the filtrate was combined with an equal volume of thiobarbituric acid (TBA) reagent. Then put in a water bath at 95 °C for 30 min. The absorbance at 532 nm was measured after cooling to room temperature, using a UV–Vis spectrophotometer. MDA concentration was found by using a standard calibration curve prepared from known MDA solutions (mg MDA/ kg of meat). This expression is consistent with standardized TBARS reporting guidelines for meat systems and represents the amount of lipid peroxidation products formed during storage.

TBARS Calculation

TBARS (mg MDA/kg) =($A \times k \times V$) / m sample

Where:

A = absorbance at 532 nm

k = conversion factor obtained from MDA calibration curve (mg MDA/mL per absorbance unit)

V = volume of the filtrate used in the reaction (mL)

m□a□□□e = sample mass (kg)

Color Determination (CIE L*, a*, b*)

Instrumental meat color was measured using the CIE L*, a*, b* system with a calibrated colorimeter set to illuminant D65, and observer 10°. After equilibration, red L* (lightness), a* (redness) and b* (yellowness) on the surface of sample. Readings were recorded (average of three locations per sample). Changes in a* and b* values were monitored to assess surface discoloration over storage day [16].

Microbial accounts

Microbial load of meat samples was measured depending on the procedures of the American Public Health Association (APHA). placed 10 g of meat into sterile stomacher bags, and mixed with 90 mL of sterile buffered peptone water for 2 minutes. After Preparing serial folds dilutions, aliquots were placed on agar media for microbial enumeration of target microbial groups.

Total Plate Count (TPC) was recorded through spreading appropriate dilutions onto Plate Count Agar, followed by incubation process at 30 °C for 48 h. *Psychrotrophic* bacteria were applied on the same medium and incubated at 4 °C for 7 days. *Coliforms* were counted by the use of Violet Red Bile Agar (VRBA) after incubation at 37 °C for 24 h. All microbial account results were expressed as log CFU/g of meat.

Fatty acid analysis

1. Lipid Extraction (Folch Method)

Total lipids were extracted from samples by following the Folch procedure [28]. 10 g of minced meat were mixed with chloroform : methanol (2:1, v/v), with adding a salt solution to promote the separation of their phases. The organic phase (containing total lipids) was collected and evaporated to dry

ISSN 2072-3857

under nitrogen prior to further fatty acids analysis.

2. Fatty Acid Methyl Ester (FAME) Preparation

Fatty acid methyl esters (FAMEs) were prepared from dried lipids using a two step of alkaline/acidic methylation procedure. Prior to methylation, an internal standard (C19:0 methyl ester) was added to lipid extract to monitor analytical recovery and enable calculation of individual fatty acids as a proportion of total identified FAMEs. Lipids were first saponified using 0.5 N NaOH in methanol under heating, then esterified with boron trifluoride–methanol (BF□, 14%) to convert both free and bound fatty acids into FAMEs. After methylation, the addition of hexane and distilled water enabled the collection of the upper hexane layer containing FAMEs for GC analysis.

3. GC-FID Analysis

FAMEs were analyzed by gas chromatography with flame-ionization detector (GC-FID) and a highly polar capillary column such as SP-2560 (100 m × 0.25 mm, 0.2 μm film). The injector was maintained at 250 °C, and the oven was operated under a controlled temperature ramp to achieve high-resolution separation of saturated, monounsaturated, polyunsaturated, and cis/trans isomers. Fatty acids were identified by comparing the retention times of sample peaks with those of FAME standards. Individual fatty acids were expressed as a percentage of total identified fatty acids (% of total FA). Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were calculated by summing the respective groups, and selected fatty acids (C18:2 n-6, C18:3 n-3, CLA) were reported individually.

4. Health Lipid Indices Calculation

Nutritional lipid quality indices were calculated from the chromatographically determined fatty acid profile following the methodology described by Aberoumand and Baez [3]. The polyunsaturated-to-saturated fatty acid ratio (PUFA/SFA) and the omega-6 to omega-3 ratio (n-6/n-3) were determined by summing the respective fatty acid groups. The Atherogenic Index (AI) and Thrombogenic Index (TI) were calculated based on the relative contributions of hypercholesterolemic (C12:0, C14:0, C16:0) and protective fatty acids (MUFA, PUFA n-6, PUFA n-3). The hypocholesterolemic/hypercholesterolemic ratio (h/H) was obtained by dividing the sum of hypocholesterolemic fatty acids (MUFA + PUFA) by the sum of hypercholesterolemic fatty acids (C14:0 + C16:0). All indices were computed for each sample to evaluate the effects of treatments on the nutritional and cardiovascular implications of the meat lipid profile.

Statistical Analysis

Data were analyzed using XLSTAT [4]. Factorial Completely Randomized Design (CRD), a two-way ANOVA was conducted to evaluate the effects of treatment (control, 1%, 2%, and 3% green walnut hull extract) and storage time (Day 1, 3, 6, and 9), including their interaction, and mean separation was carried out using Duncan's Multiple Range Method (DMRM) whenever significant differences were detected (5% and 1%) [34]. Microbial data were log□□-transformed before analysis.

Results

Total Phenolic Content (TPC)

The TPC of the green walnut hull extract was 38.2 mg GAE/g DM, reflecting a notably high concentration of extractable phenolic compounds obtained under the

ISSN 2072-3857

applied hydroethanolic ultrasound-assisted extraction conditions.

Lipid Oxidation Measurement (TBARS as MDA)

Thiobarbituric acid reactive substances (TBARS) increased progressively ($p \leq 0.01$) in all treatments as storage time progressed from 1 to 9 days (Table 1). On day 9 the control samples (T1) exhibited the highest TBARS (~1.14 mg MDA/kg), indicating substantial lipid oxidation, while extract treated meat samples with green walnut husk extract showed significantly lower MDA levels. Each treatment group experienced a significant increase in TBARS over time, yet the walnut husk extract treated samples consistently maintained lower oxidation rates, T4 exhibited the lowest value (0.480 ± 0.015 mg MDA/kg) at storage day 3, while the highest value was in T1(0.638 ± 0.010 mg MDA/kg). in addition, the lowest TBARS values were recorded in T3 (0.772 ± 0.013 and 0.932 ± 0.026 mg MDA/kg at 6 and 9 days of storage respectively) and T4 (0.735 ± 0.026 and 0.883 ± 0.012 mg MDA/kg respectively for the corresponding periods) as compared with the highest value in T1 (0.866 ± 0.024 and 1.143 ± 0.007 mg MDA/kg respectively at the corresponding times). These results demonstrate that green walnut husk extract effectively retarded the development of oxidative rancidity in the meat during cold storage.

Treatment	TBARS (mg MDA/kg meat) Storage periods (days)			
	1	3	6	9
T_1	^{d} 0.215 ± 0.017^{A}	^{c} 0.638 ± 0.010^{A}	^{b} 0.866 ± 0.024^{A}	^{a}1.143 ± 0.007^{A}
T_2	^{d} 0.195 ± 0.003^{A}	^{c} 0.632 ± 0.011^{A}	^{b} 0.843 ± 0.024^{AB}	^{a}1.073 ± 0.042^{A}
T_3	^{d} 0.186 ± 0.003^{A}	^{c} 0.552 ± 0.027^{B}	^{b} 0.772 ± 0.013^{BC}	^{a} 0.932 ± 0.026^{B}
T_4	^{d} 0.185 ± 0.003^{A}	^{c} 0.480 ± 0.015^{C}	^{b} 0.735 ± 0.026^{C}	^{a} 0.883 ± 0.012^{B}

Table 1. Effects of green walnut hull extract on lipid oxidation in *semimembranosus* muscle of Karadi lambs during refrigerated storage (Mean±SE)

- Means with different uppercase letters reflect significant differences ($p \leq 0.01$) between treatments for each period.
- Means with different lowercase letters reflect significant differences ($p \leq 0.01$) between periods for each treatment.

Color Analysis (CIE L*, a*, b*)

Walnut shell extract affected significantly on the meat color attributes (L*, a*, b*, chroma, and hue) throughout refrigerated storage (Table 2). At day 1, the lightest and saturated color, higher L* (lightness), a* (redness), b* (yellowness), and chroma values recorded in T4 in comparison with the control (T1) and other treatments ($p \leq 0.01$). lightness (L*) dropped more in control meat than in treated meat over time, which that meaning the control became darker during storage. After 9 days, control L* fell to 33.797 ± 0.068, while T4 remained at 37.766 ± 0.100, a significant difference ($p \leq 0.01$). This proved that the walnut extract helped maintenance meat

ISSN 2072-3857

brighter appearance, may be by reducing oxidative color browning and moisture loss. The initial high redness (a*) was in T4 (16.686 ± 0.096) versus low in the control (14.985 ± 0.078), indicating that walnut extract improved the red color intensity of the meat. All treatments experienced a gradual decline in L*, a*, b*, and chroma with storage time increased from 1 to 9 days (each period compared with the previous one was significantly different at p≤0.01).Whereas, the rate of decline proceeded more slowly in samples treated with walnut extract. By day 9, T4 retained a higher a* and chroma (15.722 ± 0.058 and 19.675 ± 0.084, respectively) than the control (12.722 ± 0.061 and 16.014 ± 0.083 for the same traits respectively), demonstrating better color retention with higher extract levels. Similarly, the treated samples exhibit more yellowness (b*). Which followed the same trend and stayed highest in T4 throughout storage (12.816 ± 0.047, 12.526 ± 0.055, 12.121 ± 0.070 and 11.829 ± 0.063 at storge periods 1,3,6 and 9 days respectively) compared to other treatments, which indicating that the treated meat kept a more vivid overall color. The hue angle (°H) behaved differently: the control's hue increased over time (from ~37.08° to 37.40°), indicating a shift toward more brownish, less red color. In contrast, T4 showed a slight decrease in hue (from 37.527 ± 0.070° to 36.956 ± 0.046°) (p≤0.01), implying it remained more red-hued. As a result, higher concentrations of walnut shell extract effectively slowed down color deterioration; treated meat samples remained lighter, redder, and more color saturated than untreated meat during refrigerate storage.

Table 2. Effects of green walnut hull extract on color characteristics of Karadi lamb *semimembranosus* muscle during refrigerated storage (Mean ± SE).

Treatment	***L****			
	Storage periods (days)			
	1	**3**	**6**	**9**
T1	^{a} 36.925±0.140 ^{D}	^{b} 35.716 ± 0.102 ^{D}	^{c} 34.716 ± 0.049 ^{D}	^{d} 33.797 ± 0.068 ^{D}
T2	^{a} 37.993±0.122 ^{C}	^{b} 37.345 ± 0.101^{C}	^{c} 36.704 ± 0.068 ^{C}	^{d} 35.978 ± 0.090 ^{C}
T3	^{a} 38.785±0.147 ^{B}	^{b} 38.154 ± 0.078 ^{B}	^{c} 37.713 ± 0.051 ^{B}	^{d} 37.159 ± 0.020 ^{B}
T4	^{a} 39.263±0.112 ^{A}	^{b} 38.663 ± 0.066 ^{A}	^{c} 38.219 ± 0.067 ^{A}	^{d} 37.766 ± 0.100 ^{A}
Treatment	***a****			
	Storage periods (days)			
	1	**3**	**6**	**9**
T1	^{a}14.985±0.078 ^{D}	^{b}14.216 ± 0.050 ^{D}	^{c}13.416 ± 0.058 ^{D}	^{d}12.722 ± 0.061 ^{D}
T2	^{a} 15.587±0.084 ^{C}	^{b}15.083 ± 0.090 ^{C}	^{c}14.591 ± 0.090 ^{C}	^{d}14.118 ± 0.064 ^{C}
T3	^{a} 16.289±0.099 ^{B}	^{b}15.895 ± 0.075 ^{B}	^{c}15.426 ± 0.066 ^{B}	^{d}15.007 ± 0.064 ^{B}
T4	^{a} 16.686±0.096 ^{A}	^{b}16.396 ± 0.082 ^{A}	^{c} 16.011 ± 0.061	^{d}15.722 ± 0.058

ISSN 2072-3857

			A	A
Treatment	***b****			
	Storage periods (days)			
	1	**3**	**6**	**9**
T1	a 11.325 ± 0.071 D	b 10.819 ± 0.047 D	c 10.232 ± 0.054 D	d 9.726 ± 0.058 D
T2	a 11.819 ± 0.050 C	b 11.419 ± 0.052 C	c 11.012 ± 0.066 C	d 10.625 ± 0.066 C
T3	a 12.425 ± 0.053 B	b 12.005 ± 0.060 B	c 11.520 ± 0.049 B	d 11.226 ± 0.066 B
T4	a 12.816 ± 0.047 A	b 12.526 ± 0.055 A	c 12.121 ± 0.070 A	d 11.829 ± 0.063 A
Treatment	***Chroma***			
	Storage periods (days)			
	1	**3**	**6**	**9**
T1	a 18.783 ± 0.104 D	b 17.864 ± 0.068 D	c 16.873 ± 0.080 D	d 16.014 ± 0.083 D
T2	a 19.561 ± 0.095 C	b 18.918 ± 0.102 C	c 18.280 ± 0.111 C	d 17.669 ± 0.092 C
T3	a 20.487 ± 0.109 B	b 19.919 ± 0.097 B	c 19.252 ± 0.082 B	d 18.741 ± 0.090 B
T4	a 21.039 ± 0.104 A	b 20.633 ± 0.097 A	c 20.082 ± 0.091 A	d 19.675 ± 0.084 A
Treatment	***Hue***			
	Storage periods (days)			
	1	**3**	**6**	**9**
T1	c 37.080 ± 0.031 C	b 37.272 ± 0.025 AB	ab 37.331 ± 0.032 A	a 37.398 ± 0.032 A
T2	a 37.171 ± 0.047 BC	a 37.128 ± 0.038 BC	a 37.043 ± 0.060 B	a 36.965 ± 0.048 B
T3	a 37.337 ± 0.068 B	b 37.064 ± 0.046 C	c 36.751 ± 0.012 C	c 36.797 ± 0.052 C
T4	a 37.527 ± 0.070 A	a 37.381 ± 0.067 A	b 37.125 ± 0.053 B	b 36.956 ± 0.046 B

- Means with different uppercase letters reflect significant differences ($p \leq 0.01$) between treatments for each period.
- Means with different lowercase letters reflect significant differences ($p \leq 0.01$) between periods for each treatment.

Microbial accounts

Total Plate Count (TPC): Initial TPC values were low and similar across all treatments (Table 3). Over 9 days, TPC rose markedly in every sample ($p \leq 0.01$), but most slowly in higher-extract treatments.

ISSN 2072-3857

For example, by day 9 the control (T1) TPC reached 6.981 ± 0.021 log CFU/g whereas the T4 was much lower (5.803 ± 0.012 log CFU/g, $p \leq 0.01$), with intermediate values in T2 and T3 (6.615 ± 0.020 and 6.193 ± 0.012 log CFU/g, respectively), all treatments differed significantly from one another within the same storage period. Likewise, within each treatment the counts increased significantly over time ($p \leq 0.01$) in all samples. Overall, higher extract concentrations consistently suppressed TPC: at each sampling day, T4 < T3 < T2 < T1, differences with $p \leq 0.01$ between them. According to the Iraqi Central Organization for Standardization and Quality Control (COSQC) [9], 5 log CFU/g the good-quality level for aerobic plate count in chilled meat while 6 log CFU/g is the maximum acceptable level. Accordingly, T1, T2, and T3 exceeded the maximum acceptable limit on day 9 (6.981, 6.615, and 6.193 log CFU/g, respectively), whereas T4 was below the maximum permissible level at 5.803 log CFU/g.

Psychrotrophic bacteria: Psychrotrophic counts followed a similar pattern. All samples started with low psychrotrophic levels and increased significantly ($p \leq 0.01$) with storage. By day 9, T1 recorded the highest count (6.512 ± 0.021 log CFU/g, $p \leq 0.01$), while T4 had the lowest (5.366 ± 0.015 log CFU/g), T2 and T3 had intermediate counts (6.139 ± 0.015 and 5.735 ± 0.014 log CFU/g, respectively). Within each treatment, psychrotrophic counts at day 9 significantly exceeded those at day 1 and those at day 3 and 6 ($p \leq 0.01$). Thus, A clear concentration-dependent reduction in psychrotrophic counts was observed, with higher extract concentrations (particularly T4) consistently resulting in lower bacterial loads at all storage periods. All treatments in the present study remained below the spoilage threshold through day 9, although T1 approached it most closely. T4 showed the greatest inhibitory effect, remaining approximately 1.63 log units below the 7-log threshold which used the limit for acceptable meat quality for psychrotrophic bacteria [33]

Coliforms: Coliform counts were lowest overall but followed similar patterns of those of TPC and psychrotrophic bacteria. All treatments had negligible coliforms counts at day 1 (near detection limits), which increased by day 9. T1 reached the highest coliform count (4.733 ± 0.021 log CFU/g, $p \leq 0.01$), whereas T4 again was the lowest (3.698 ± 0.046), with T2 and T3 between (4.330 ± 0.013 and 3.941 ± 0.015 log CFU/g, respectively). Coliform levels increased significantly from day 1 to day 9 within each treatment ($p \leq 0.01$). These results demonstrate that walnut green husk extract inhibited coliform growth in a dose dependent pattern showing significant differences ($p \leq 0.01$) between treatments.

Harmful microbial loads became evident by day 6, with an increase by day 9, which the highest microbial load occurred in the control group.

ISSN 2072-3857

Table 3. Effects of green walnut hull extract on microbial quality of Karadi lamb *semimembranosus* muscle during refrigerated storage (Mean ± SE).

Treatment	Total Plate Count (log CFU/g)			
	Storage periods (days)			
	1	3	6	9
T1	$^{d}_{A}$ 3.712 ± 0.012	^{c} 4.697 ± 0.014 ^{A}	^{b} 5.726 ± 0.011 ^{A}	$^{a}_{A}$ 6.981 ± 0.021
T2	$^{d}_{B}$ 3.402 ± 0.013	^{c} 4.398 ± 0.011 ^{B}	^{b} 5.414 ± 0.011 ^{B}	$^{a}_{B}$ 6.615 ± 0.020
T3	$^{d}_{C}$ 3.219 ± 0.012	^{c} 4.212 ± 0.010 ^{C}	^{b} 5.234 ± 0.012 ^{C}	$^{a}_{C}$ 6.193 ± 0.012
T4	$^{d}_{D}$ 2.998 ± 0.010	^{c} 3.997 ± 0.013 ^{D}	^{b} 5.011 ± 0.011 ^{D}	$^{a}_{D}$ 5.803 ± 0.012
Treatment	Psychotropic (log CFU/g)			
	Storage periods (days)			
	1	3	6	9
T1	$^{d}_{A}$ 2.985 ± 0.012	^{c} 4.007 ± 0.015 ^{A}	^{b} 5.001 ± 0.013 ^{A}	$^{a}_{A}$ 6.512 ± 0.021
T2	$^{d}_{B}$ 2.799 ± 0.009	^{c} 3.810 ± 0.010 ^{B}	^{b} 4.810 ± 0.008 ^{B}	$^{a}_{B}$ 6.139 ± 0.015
T3	$^{d}_{C}$ 2.608 ± 0.009	^{c} 3.622 ± 0.010 ^{C}	^{b} 4.620 ± 0.008 ^{C}	$^{a}_{C}$ 5.735 ± 0.014
T4	$^{d}_{D}$ 2.430 ± 0.009	^{c} 3.438 ± 0.011 ^{D}	^{b} 4.440 ± 0.010 ^{D}	$^{a}_{D}$ 5.366 ± 0.015
Treatment	Coliforms (log CFU/g)			
	Storage periods (days)			
	1	3	6	9
T1	$^{d}_{A}$ 2.012 ± 0.012	^{c} 3.041 ± 0.013 ^{A}	^{b} 4.029 ± 0.013 ^{A}	$^{a}_{A}$ 4.733 ± 0.021
T2	$^{d}_{B}$ 1.831 ± 0.010	^{c} 2.853 ± 0.010 ^{B}	^{b} 3.845 ± 0.009 ^{B}	$^{a}_{B}$ 4.330 ± 0.013
T3	$^{d}_{C}$ 1.669 ± 0.009	^{c} 2.686 ± 0.009 ^{C}	^{b} 3.680 ± 0.009 ^{C}	$^{a}_{C}$ 3.941 ± 0.015
T4	$^{d}_{D}$ 1.512 ± 0.010	^{c} 2.528 ± 0.010 ^{D}	^{b} 3.521 ± 0.009 ^{D}	$^{a}_{D}$ 3.698 ± 0.046

- Means with different uppercase letters reflect significant differences ($p \leq 0.01$) between treatments for each period.
- Means with different lowercase letters reflect significant differences ($p \leq 0.01$) between periods for each treatment.

Major fatty acids

Table 4 showed the major fatty acid of Karadi lamb *semimembranosus* muscle as

ISSN 2072-3857

affected by walnut green husk extract during refrigerated storage. At day 1, no significant differences ($p \leq 0.01$) recorded in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA), confirming similar initial lipid profiles across all samples

After 9 days of storage, SFA levels increased significantly within each treatment ($p \leq 0.01$). T1 and T2 exhibited the highest SFA value (40.893 ± 0.131% and 40.471 ± 0.079 %, respectively), while T3 and T4 showed lowest SFA percentages (39.905 ± 0.190 % and 39.606 ± 0.144 %, respectively).

MUFA content remained statistically unchanged throughout storage, with no significant differences detected either between storage periods or among treatments ($p > 0.01$). MUFA values were stable at approximately 41% across all treatments at both day 1 and day 9.

In contrast, PUFA content showed a significant reduction during storage in all treatments ($p \leq 0.01$). However, at day 9 the magnitude of PUFA loss was significantly ($p \leq 0.01$) influenced by walnut green husk extract concentration. T1 showed the lowest PUFA percentage (17.665 ± 0.077%), whereas PUFA retention increased progressively with extract concentration, reaching the highest value in T4 (19.033 ± 0.044%).

Table 4. Effects of walnut green husk extract on major fatty acids of Karadi lamb *semimembranosus* muscle during refrigerated storage (Mean ± SE).

Storage Period (days)	**SFA%**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	^{b} 38.864 ± 0.052^{A}	^{b} 38.923 ± 0.071^{A}	^{b} 38.863 ± 0.072^{A}	^{b} 38.860 ± 0.091^{A}
9	^{a} 40.893 ± 0.131^{A}	^{a} 40.471 ± 0.079 ^{A}	^{a} 39.905 ± 0.190 ^{B}	^{a} 39.606 ± 0.144 ^{B}
Storage Period (days)	**MUFA%**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	^{a} 41.259 ± 0.091^{A}	^{a} 41.069 ± 0.117^{A}	^{a} 41.132 ± 0.065 ^{A}	^{a} 41.194 ± 0.113 ^{A}
9	^{a} 41.442 ± 0.070 ^{A}	^{a} 41.440 ± 0.072 ^{A}	^{a} 41.471 ± 0.212 ^{A}	^{a} 41.361 ± 0.134 ^{A}
Storage Period (days)	**PUFA%**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	^{a} 19.877 ± 0.056^{A}	^{a} 20.007 ± 0.081^{A}	^{a} 20.005 ± 0.055 ^{A}	^{a} 19.946 ± 0.027 ^{A}
9	^{b} 17.665 ± 0.077 ^{D}	^{b} 18.089 ± 0.016 ^{C}	^{b} 18.625 ± 0.052 ^{B}	^{b} 19.033 ± 0.044 ^{A}

- Means with different uppercase letters reflect significant differences ($p \leq 0.01$) between treatments for each period.
- Means with different lowercase letters reflect significant differences ($p \leq 0.01$) between periods for each treatment.

ISSN 2072-3857

Individual Polyunsaturated Fatty Acids

The effects of walnut green husk extract on linoleic acid (C18:2 n-6), α-linolenic acid (C18:3 n-3), and conjugated linoleic acid (CLA) in Karadi lamb semimembranosus muscle during storage are presented in Table 5. At day 1, no significant differences ($p \leq 0.01$) were observed among treatments for any of the fatty acids, indicating a comparable initial fatty acid composition across all samples.

By day 9 of refrigerated storage, the contents of C18:2 n-6, C18:3 n-3, and CLA in meat decreased significantly within all treatments ($p \leq 0.01$). Despite, the overall decrease the degree of reduction varied significantly among treatments ($p \leq 0.01$), with extract-treated samples retained higher levels than the control. For linoleic acid (C18:2 n-6), T1 showed the lowest value (12.208 ± 0.025%), whereas the highest level was recorded in T4 (12.951 ± 0.020%), followed by T3 (12.673 ± 0.022%) and T2 (12.405 ± 0.016%). Similarly, α-linolenic acid (C18:3 n-3) was lowest in T1 (1.990 ± 0.012%) and highest in T4 (2.247 ± 0.012%). Conjugated linoleic acid (CLA) exhibited the same pattern, ranging from 1.967 ± 0.015% in T1 to 2.260 ± 0.018% in T4 at day 9, demonstrating improved preservation of individual PUFA fractions with increasing walnut green husk extract concentration.

Table 5. Effects of walnut green husk extract on individual polyunsaturated fatty acids of Karadi lamb *semimembranosus* muscle during refrigerated storage (Mean ± SE).

Storage Period (days)	**C18_2n6 %**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	^{a} 14.024 ± 0.027 ^{A}	^{a} 14.016 ± 0.024 ^{A}	^{a} 14.021 ± 0.019 ^{A}	^{a} 14.023 ± 0.026 ^{A}
9	^{b} 12.208 ± 0.025 ^{D}	^{b}12.405 ± 0.016 ^{C}	^{b} 12.673 ± 0.022 ^{B}	^{b} 12.951 ± 0.020 ^{A}
Storage Period (days)	**C18_3n3 %**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	^{a} 3.013 ± 0.017 ^{A}	^{a} 3.018 ± 0.012 ^{A}	^{a} 3.010 ± 0.009 ^{A}	^{a} 3.014 ± 0.008 ^{A}
9	^{b} 1.990 ± 0.012 ^{D}	^{b} 2.057 ± 0.009 ^{C}	^{b} 2.144 ± 0.008 ^{B}	^{b} 2.247 ± 0.012 ^{A}
Storage Period (days)	**CLA %**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	^{a} 2.957 ± 0.039 ^{A}	^{a} 2.961 ± 0.016 ^{A}	^{a} 2.963 ± 0.014 ^{A}	^{a} 2.952 ± 0.014 ^{A}
9	^{b} 1.967 ± 0.015 ^{D}	^{b} 2.044 ± 0.013 ^{C}	^{b} 2.148 ± 0.019 ^{B}	^{b} 2.260 ± 0.018 ^{A}

- Means with different uppercase letters reflect significant differences ($p \leq 0.01$) between treatments for each period.
- Means with different lowercase letters reflect significant differences ($p \leq 0.01$) between periods for each treatment.

ISSN 2072-3857

Nutritional lipid quality

The influence of walnut green husk extract on lipid quality indices of lamb meat throughout refrigerated storage is shown in Table 6. No significant differences among treatments were detected on day 1 ($p>0.01$) for PUFA/SFA ratio, n-6/n-3 ratio, atherogenic index (AI), thrombogenic index (TI), or hypocholesterolemic/hypercholesterolemic ratio (h/H), indicating a comparable initial lipid nutritional quality across all samples.

After 9 days of storage, the PUFA/SFA ratio decreased significantly in all treatments ($p\leq0.01$). T1 exhibited the lowest value (0.432 ± 0.003), whereas the highest PUFA/SFA ratio was recorded in T4 (0.481 ± 0.003), followed by T3 (0.467 ± 0.003) and T2 (0.447 ± 0.001).

The n-6/n-3 ratio increased significantly throughout storage in all treatments ($p\leq0.01$). At day 9, T1 showed the highest ratio (7.126 ± 0.056), while T4 recorded the lowest value (6.771 ± 0.040).

Both AI and TI values increased significantly during storage ($p\leq0.01$), reflecting a decline in lipid nutritional quality over time. Nevertheless, at day 9 The lowest AI (0.391 ± 0.002) and TI (1.125 ± 0.007) values were observed in T4, whereas T1 (AI: 0.412 ± 0.002; TI: 1.205 ± 0.006) recorded the highest values.

The h/H ratio decreased significantly throughout storage treatments ($p\leq0.01$). At day 9, T3 and T4 maintained the highest h/H ratios (4.126 ± 0.033 and 4.184 ± 0.028, respectively), compared to the lowest ratio in T1 and T2 (3.968 ± 0.016 and 4.033 ± 0.012, respectively) indicating better preservation of hypocholesterolemic fatty acids.

Table 6. Nutritional lipid quality indices of Karadi lamb *semimembranosus* muscle as affected by walnut green husk extract during refrigerated storage (Mean ± SE).

Storage Period (days)	**PUFA/SFA**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	^{a} 0.511 ± 0.001 ^{A}	^{a} 0.514 ± 0.002 ^{A}	^{a} 0.515 ± 0.002 ^{A}	^{a} 0.513 ± 0.001 ^{A}
9	^{b} 0.432 ± 0.003 ^{D}	^{b} 0.447 ± 0.001 ^{C}	^{b} 0.467 ± 0.003 ^{B}	^{b} 0.481 ± 0.003 ^{A}
Period (days)	**n-6/n-3**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	^{b} 5.637 ± 0.044 ^{A}	^{b} 5.626 ± 0.028 ^{A}	^{b} 5.642 ± 0.030 ^{A}	^{b} 5.633 ± 0.041 ^{A}
9	^{a} 7.126 ± 0.056 ^{A}	^{a} 7.024 ± 0.033 ^{AB}	^{a} 6.913 ± 0.024 ^{B}	^{a} 6.771 ± 0.040 ^{C}
Storage Period (days)	**AI**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	^{b} 0.369 ± 0.001 ^{A}	^{b} 0.370 ± 0.001 ^{A}	^{b} 0.369 ± 0.001 ^{A}	^{b} 0.369 ± 0.001

ISSN 2072-3857

				[A]
9	[a] 0.412 ± 0.002 [A]	[a] 0.406 ± 0.001 [A]	[a] 0.397 ± 0.003 [B]	[a] 0.391 ± 0.002 [B]
Storage Period (days)	**TI**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	[b] 1.011 ± 0.002 [A]	[b] 1.017 ± 0.003 [A]	[b] 1.015 ± 0.002 [A]	[b] 1.014 ± 0.004 [A]
9	[a] 1.205 ± 0.006 [A]	[a] 1.180 ± 0.004 [B]	[a] 1.148 ± 0.009 [C]	[a] 1.125 ± 0.007 [D]
Storage Period (days)	**h/H**			
	Treatment			
	T1	**T2**	**T3**	**T4**
1	[a] 4.440 ± 0.015 [A]	[a] 4.419 ± 0.017 [A]	[a] 4.431 ± 0.013 [A]	[a] 4.435 ± 0.020 [A]
9	[b] 3.968 ± 0.016 [B]	[b] 4.033 ± 0.012 [B]	[b] 4.126 ± 0.033 [A]	[b] 4.184 ± 0.028 [A]

- Means with different uppercase letters reflect significant differences ($p \leq 0.01$) between treatments for each period.
- Means with different lowercase letters reflect significant differences ($p \leq 0.01$) between periods for each treatment.

Discussion

Total Phenolic Content (TPC)

The total phenolic content (TPC) of green walnut hull extracts reported in the literature varies widely, from single-digit to triple-digit values (in mg gallic acid equivalents per gram of dry material, mg GAE/g DM). This wide range results may be due to the differences in walnut genotype, maturity, and different extract procedure. Our TPC value (38.2 mg GAE/g DM) falls in the mid range of previously studies values and it is within the expected range for Juglans regia green husk extracts and aligns with previously studies with similar extract method. This measured TPC is slightly higher than Oliveira et al. [26] lowest reported value (~32.6 mg/g) and is near the lower end of the Hungarian cultivar range (44.2–57.4 mg/g) [20]. In other words, 38.2 mg/g is comparable to a mid-performing conventional extraction: above the poorest yields and within the lower part of the higher-yield range from previous studies.

Lipid Oxidation Measurement (TBARS)

The green walnut hull extract exhibited a strong antioxidant effect in the meat system, as evidenced by the significant reduction in TBARS during refrigerated storage. This effect is mainly attributed to the extract's high total phenolic content (38.2 mg GAE/g DM), since phenolic compounds are known to inhibit lipid oxidation through hydrogen donation to lipid radicals and chelation of pro-oxidant metal ions [37]. Accordingly, treated samples showed consistently lower lipid peroxidation compared with the control.

The present findings are in agreement with previous studies reporting enhanced oxidative stability in meat and fish products

supplemented with walnut green husk extracts. Salejda et al. [30] mentioned that lipid oxidation inhibited and extended meat shelf life when treated meat with walnut green husks. These results confirm the effectiveness of walnut-derived phenolics across different muscle food systems.

Overall, immersion of lamb meat in green walnut hull extract effectively reduced lipid oxidation throughout the 9 days storage period, which supporting its potential application as a natural antioxidant in preserving meat quality, consistent with previous studies on walnut husk strong bioactivity [30,37].

Color Analysis (CIE L*, a*, b*)

Green walnut husk extract slowed the usual color deterioration of refrigerated lamb meat. The lower lightness (L*) in treated samples due to the natural dark pigments in the husk, as Salejda et al. [30] also observed that adding walnut husk decreased lightness of sausages. More importantly, redness and color saturation were better preserved by the extract treatments. Control lost their red color rapidly, whereas immersion samples in green walnut husk extract kept a* and chroma at higher levels. This is consistent with previous research support that meat color instability was smaller throughout chilled storage with applied of walnut husk [30]. That is, the treated meat showed less color change to dull brown (smaller hue change) over storage time.

These effects can be related to the present strong antioxidant compounds in green walnut husk extract. In the present work, the immersion meat in green walnut husk extracts inhibited malondialdehyde (MDA) formation comparing with the control, which improved oxidative stability. Plant polyphenols sources are proven to act as effective free radical scavengers, thereby interrupting lipid peroxidation chain reactions, which limiting the accumulation of secondary oxidation products such as MDA [40]. Due to the fact that lipid oxidation and myoglobin oxidation closely interconnected processes in red meat, the reduction of lipid derived reactive compounds contributes significantly to meat color stability. Lipid oxidation products (MDA and other aldehydes) accelerate oxymyoglobin oxidation to metmyoglobin, that leading to discoloration and browning of meat [15, 40]. Therefore, by suppressing lipid oxidation, green walnut husk extracts antioxidants compounds indirectly decrease myoglobin oxidation, which maintain the red oxymyoglobin form and resulting in higher redness (a*) and color saturation during refrigerated storage. These indicate that oxidative stability maintenance is a key mechanism enhanced color preservation in extract immersion meat. As a result, this explains why the extract□treated meat retained higher a* (redness) and chroma and exhibited smaller hue angles (less brown) during refrigerate storage.

Microbial accounts

Meat samples treated with green walnut husk extract led to reduced microbial loads throughout refrigerated storage, significant inhibitory effect associated with higher extract concentration. Total plate count (TPC), *psychrotropic*, and *coliforms* bacteria recorded lower in treated samples than the control, particularly in the T4 group. These results similar to prior studies depended on green walnut husk extract in meat preservation. Salejda et al. [30] indicated that sausage microbial growth reduced during product storage when adding green walnut husk powder, while other study reported a dose dependent reduction in microbial in trout fillets coated with walnut husk extract [23].

ISSN 2072-3857

The observed antimicrobial action can be related to the extract's rich of phenolics and other bioactive compounds. Our walnut husk extract total phenolics was 38.2 mg GAE/g DM which contributed to its antimicrobial activity. Walnut husk extract is rich in flavonoids, phenolic acids (e.g., gallic, caffeic, vanillic acids), and naphthoquinones such as juglone, which collectively contributes to its antibacterial properties [6].

Mechanistically, these compounds compromise bacterial membranes integrity, causing leakage of intracellular constitutes, disruption of metabolic function, and ultimately cell death [38]. Juglone, in particular, has been shown to induce oxidative stress and empire bacterial membrane integrity. In our treated samples, such effects likely attributed to the suppressed microbial proliferation. The dose-dependent trend observed (T4 < T3 < T2 < T1) further suggested that higher extract concentrations supplied sufficient bioactive compounds to exceed the minimum inhibitory concentration.

While the extract did not prevent bacterial growth throughout time, but it delayed spoilage progression. These results reinforce the potential of green walnut husk extract as a natural preservative that extends shelf life rather than sterilizes meat.

Fatty acids indices

Major fatty acids: During refrigeration storage, our results showed an increase in SFA as a result of the decrease in PUFA which are consequences of lipid oxidation in meat, PUFA are more prone to oxidation, and lower the relative proportion of PUFA and produce an increase in SFA percentage [11]. The current findings suggest that walnut green husk extract effectively reduced storage related changes, by preserving PUFA levels and limiting the increase in SFA.

The improved PUFA retention especially in T3 and T4 may be related to the present of strong antioxidant in the walnut green husk, such as phenolic compounds like gallic and ellagic acids, flavonoids, and naphthoquinones such as juglone [37]. These compounds may limit lipid oxidation through radical scavenging activity and chelate pro-oxidant metal ions, thereby interrupting lipid peroxidation chains and protecting unsaturated fatty acids from oxidative degradation.

The absence of significant differences in MUFA content may be associated with the higher oxidative stability of MUFA as compared with PUFA. MUFA, like oleic acid, are less prone to oxidative due to having only one double bond, whereas PUFA have multiple double bonds which are more susceptible to lipid oxidation [8,11]. Similar stability of MUFA has been reported in meat products supplemented with walnut green husk [30].

These results suggest that Walnut green husk extract preserves lipid quality in lamb meat throughout refrigeration storage by limiting both PUFA loss and unfavorable fatty acid changes, supporting its use as a natural antioxidant to enhance lipid stability and nutritional value in red meats.

Individual Polyunsaturated Fatty Acids: linoleic acid (C18:2 n-6), α-linolenic acid (C18:3 n-3), and conjugated linoleic acid (CLA) decreasing throughout refrigerated storage may be associated with PUFA susceptibility to oxidative degradation, particularly n-3 fatty acids and CLA, which are highly prone to oxidation related to their

ISSN 2072-3857

multiple double bonds, providing numerous available sites for free radical attack and promote lipid oxidation processes [11,18]. The PUFA reductions in the control samples can be related to their greater susceptibility to oxidative deterioration under refrigerated conditions. The higher retention occurs of C18:2 n-6 and C18:3 n-3 in walnut green husk extract immersion samples reflect a protective effect of them against lipid oxidation, attribute to the extract's phenolic compounds with strong antioxidant properties [37], which led to the reduce TBARS values in T3 and T4, as a result of lipid oxidation inhibition and improved stability of polyunsaturated fatty acids in extract treated meat [11].

Conjugated linoleic acid (CLA) is recognized for its effective health promoting properties, but susceptible to oxidative during storage. Previous studies indicated that CLA may undergo oxidative degradation in food, leading to its gradual decrease under oxidative conditions [24]. Thus, the decline in CLA observed throughout refrigerated storage in the present study may be related to its oxidation alongside other lipid components in meat.

The concentration dependent response observed across the three fatty acids further supports the contribution of walnut green husk phenolics to the stabilization of meat lipids. Higher extract levels likely supplied sufficient antioxidant compounds to effectively neutralize reactive oxygen species and lipid radicals, thereby slowing oxidative degradation. This finding aligns with previous reports demonstrating stronger antioxidant efficacy of walnut green husk extracts at higher concentrations [37].

Nutritional lipid quality: The decline in PUFA/SFA and the simultaneous increase in n-6/n-3, AI, and TI values during refrigerated storage reflect the extent of lipid oxidation and nutritional quality deterioration of meat lipid fraction [11]. Due to high oxidation susceptibility of polyunsaturated fatty acids during storage, resulting in unfavorable shifts in health lipid indices. The present results proved that walnut green husk extract significantly mitigated these adverse changes, especially at higher concentration levels.

A higher PUFA/SFA is recognized as indicator of a healthier lipid profile and associated with more cardiovascular risk markers, which attribute to reductions in LDL cholesterol and other risk factors in human nutrition [39]. In the present study, meat samples immersed in walnut green husk extract exhibited improved oxidative stability, highlighting the protective effect of the extract.

The lower n-6/n-3 in immersed samples further support this beneficial effect. An increase in n-6/n-3 are associated with pro inflammatory conditions and an increased cardiovascular risk [32]. Walnut green husk extract effectively reduced the loss of n-3 fatty acids, thereby moderating the increase in n-6/n-3. This effect may consistent with the strong antioxidant compounds present in walnut green husk, that protect unsaturated fatty acids from oxidative reaction [37].

The atherogenicity (AI) and thrombogenicity (TI) indices reflect the balance between saturated and unsaturated fatty acids which serve as indicators of the cardiovascular relevance of meat lipids. Lower AI and TI values associated with a healthier fatty acid profile in red meat. In this study, reduced AI and TI in walnut green husk treated samples indicate nutritional quality improvement of meat

ISSN 2072-3857

through greater retention of unsaturated fatty acids [10].

Higher h/H also obtained in treated samples which indicate greater retention of hypocholesterolemic fatty acids relative to hypercholesterolemic. These results similar with earlier studies demonstrated that walnut green husk and other phenolic rich plant extracts enhance lipid stability and preserve beneficial fatty acids in meat during storage [25, 30].

Conclusion

The actual deterioration in meat characteristics began from day 3 of refrigerated storage. Green walnut hull extract significantly improved the refrigerated storage stability of Karadi lamb semimembranosus with stronger effects at higher concentrations. Compared with the control, extract-treated samples showed lower TBARS (slower lipid oxidation), better color retention (higher L*, a*, b*, and chroma with reduced hue shift), and reduced microbial growth (TPC, psychrotrophs, and coliforms), particularly at higher concentration levels. The extract also mitigated storage-induced deterioration in lipid composition by preserving PUFA (including C18:2 n-6, C18:3 n-3, and CLA) and maintaining more favorable lipid health indices (higher PUFA/SFA and h/H; lower n-6/n-3, AI, and TI). Overall, green walnut hull extract especially at 3% (T4) is a promising clean-label additive for prolonging shelf life and preserving nutritional and quality attributes of lamb meat during refrigerated

References:

1. Abdullah, F. A. A., Horáková, M., & Kameník, J. (2024). Oxidation status and antioxidant activity of analogue meat products: TBARS, FFA, and polyphenol content. *Applied Sciences*, *14*(15), 6713. https://doi.org/10.3390/app14156713 .
2. Aberoumand, A., & Baez, C. (2023). Evaluation of fatty-acid-related nutritional quality indices in marinated fish. *Food Science & Nutrition*, *11*(3), 1686–1696. https://doi.org/10.1002/fsn3.3131.
3. Addinsoft. XLSTAT statistical software (Version 2019) [Computer software]. Paris, France: Addinsoft. https://www.xlstat.com (2019).
4. American Public Health Association (APHA). (2015). *Compendium of Methods for the Microbiological Examination of Foods* (5th ed.). APHA Press. https://doi.org/10.2105/MBEF.0222.
5. AOAC International. (2005). *Official Method 996.06: Fat (total, saturated, and unsaturated) in foods—Hydrolytic extraction gas chromatographic method*. In *Official Methods of Analysis of AOAC International* (18th ed.). AOAC International. https://pdfcoffee.com/download/41128a-aoac-official-method-99606-fat-total-saturated-and-unsaturated-in-foods-pdf-free.html.
6. Arslan, H., Koc, E. O., Ozay, Y., Canli, O., Ozdemir, S., Tollu, G., & Dizge, N. (2023). Antimicrobial and antioxidant activity of phenolic extracts from walnut (*Juglans regia* L.) green husk by using pressure-driven membrane process. *Journal of Food Science and Technology*.

ISSN 2072-3857

https://doi.org/10.1007/s13197-022-05588-w.

7. Barekat, S., Nasirpour, A., Keramat, J., Dinari, M., Meziane-Kaci, M., Paris, C., & Desobry, S. (2023). Phytochemical composition, antimicrobial, anticancer properties, and antioxidant potential of green husk from several walnut varieties (*Juglans regia* L.). *Antioxidants*, *12*(1), 52. https://doi.org/10.3390/antiox12010052
8. Booyens, K. E. (2012). Fatty acid composition and oxidative stability of lamb meat. South African Journal of Animal Science, 42(5), 517–526. https://www.scielo.org.za/scielo.php?pid=S0375-15892012000500008&script=sci_arttext.
9. Central Organization for Standardization and Quality Control (COSQC). (2023). Iraqi Quality Standard IQS 2270-4:2023, Microbial limits in food: Meat, eggs and their products, *Table 4.1*.
10. Dal Bosco, A., Cavallo, M., Menchetti, L., Angelucci, E., Cartoni Mancinelli, A., Vaudo, G., Marconi, S., Camilli, E., Galli, F., Castellini, C., & Mattioli, S. (2024). *The Healthy Fatty Index allows for deeper insights into the lipid composition of foods of animal origin when compared with the atherogenic and thrombogenicity indexes*. Foods, 13(10), 1568. https://doi.org/10.3390/foods13101568.
11. Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, *8*(10), 429. https://doi.org/10.3390/antiox8100429
12. Domínguez-López, I., Pérez, M., & Lamuela-Raventós, R. M. (2024). Total (poly)phenol analysis by the Folin–Ciocalteu assay as an anti-inflammatory biomarker in biological samples. Critical Reviews in Food Science and Nutrition, 64(27), 10048–10054. https://doi.org/10.1080/10408398.2023.2220031.
13. Efenberger-Szmechtyk, M., Nowak, A., & Czyżowska, A. (2021). Plant extracts rich in polyphenols: Antibacterial agents and natural preservatives for meat and meat products. *Critical Reviews in Food Science and Nutrition*, *61*(1), 149–178. https://doi.org/10.1080/10408398.2020.1722060.
14. Estévez, M. (2021). Critical overview of the use of plant antioxidants in the meat industry: Opportunities, innovative applications and future perspectives. *Meat Science*, *181*, 108610. https://doi.org/10.1016/j.meatsci.2021.108610.
15. Faustman, C., Sun, Q., Mancini, R., & Suman, S. P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Science*, *86*(1), 86–94. https://doi.org/10.1016/j.meatsci.2010.04.025.
16. Feng, C.-H., Arai, H., & Rodríguez-Pulido, F. J. (2024). Colour analysis of sausages stuffed with modified casings added with citrus peel extracts using hyperspectral imaging combined with multivariate analysis.

ISSN 2072-3857

Sustainability, 16(19), 8683. https://doi.org/10.3390/su16198683

17. HosseiniHashemi, S. K., Baseri, H., Mehjabin, J. J., Alam, M. K., HossinAshrafi, S. K., & Lotfi, M. (2025). Phytochemical profile and antioxidant activity of *Juglans regia* L. green husk extracts from Tuyserkan region, Iran. *BioResources, 20*(3), 7265–7277. https://doi.org/10.15376/biores.20.3.7265-7277.
18. Huang, X., et al. (2019). *Lipid oxidation and its implications to meat quality and human health. Frontiers in Nutrition*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6811465/.
19. Jahanban-Esfahlan, A., Ostadrahimi, A., Tabibiazar, M., & Amarowicz, R. (2019). A comprehensive review on the chemical constituents and functional uses of walnut (*Juglans* spp.) husk. *International Journal of Molecular Sciences, 20*(16), 3920. https://doi.org/10.3390/ijms20163920.
20. Jahanban-Esfahlan, A., Ostadrahimi, A., Tabibiazar, M., & Amarowicz, R. (2019). A comparative review on the extraction, antioxidant content and antioxidant potential of different parts of walnut (*Juglans regia* L.) fruit and tree. *Molecules, 24*(11), 2133. https://doi.org/10.3390/molecules24112133
21. Manessis, G., Kalogianni, A. I., Lazou, T., Moschovas, M., Bossis, I., & Gelasakis, A. I. (2020). Plant-derived natural antioxidants in meat and meat products. *Antioxidants, 9*(12), 1215. https://doi.org/10.3390/antiox9121215
22. Martínez-Zamora, L., Ros, G., & Nieto, G. (2020). Synthetic vs. Natural Hydroxytyrosol for Clean Label Lamb Burgers. *Antioxidants, 9*(9), 851. https://doi.org/10.3390/antiox909085.
23. Mozaffari, P., Pashangeh, S., Berizi, E., Majlesi, M., Hosseinzadeh, S., Salehi, S. O., Derakhshan, Z., & Giannakis, S. (2022). Potential of nanochitosan coating combined with walnut green husk to improve the preservation of rainbow trout (*Oncorhynchus mykiss*) during refrigerated storage. *Environmental Research, 214*, 114019. https://doi.org/10.1016/j.envres.2022.114019.
24. Mungure, T. E., & others. (2020). *Conjugated linoleic acid (CLA) and cholesterol oxidative products in food systems*. Foods, 9**(**1), 43. https://doi.org/10.3390/foods9010043.
25. Nikmaram, N., Budaraju, S., Barba, F. J., Lorenzo, J. M., Cox, R. B., Mallikarjunan, K., & Roohinejad, S. (2018). *Application of plant extracts to improve the shelf-life, nutritional and health-related properties of ready-to-eat meat products*. Meat Science, 145, 245–255. https://doi.org/10.1016/j.meatsci.2018.06.031
26. Oliveira, I., Sousa, A., Ferreira, I. C. F. R., Bento, A., Estevinho, L., & Pereira, J. A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food and Chemical Toxicology, 46*(7), 2326–2331. https://doi.org/10.1016/j.fct.2008.03.017.

ISSN 2072-3857

27. Olvera-Aguirre, G., Piñeiro-Vázquez, Á.T., & Vargas-Bello-Pérez, E. et al. (2023). Using Plant-Based Compounds as Preservatives for Meat Products: A Review. *Heliyon*, *9*(6), e17071. https://doi.org/10.1016/j.heliyon.2023.e17071.
28. Pérez-Palacios, T., Ruiz, J., Ferreira, I. M. P. L. V. O., Petisca, C., & Antequera, T. (2012). Effect of solvent to sample ratio on total lipid extracted and fatty acid composition in meat products within different fat content. *Meat Science*, *91*(3), 369–373. https://doi.org/10.1016/j.meatsci.2012.02.021.
29. Purgatorio, C., Serio, A., Chaves-López, C., Rossi, C., & Paparella, A. (2022). An overview of the natural antimicrobial alternatives for sheep meat preservation. *Comprehensive Reviews in Food Science and Food Safety*, *21*(5), 4210–4250. https://doi.org/10.1111/1541-4337.13004.
30. Salejda, A. M., Janiewicz, U., Korzeniowska, M., Kolniak-Ostek, J., & Krasnowska, G. (2016). Effect of walnut green husk addition on some quality properties of cooked sausages. LWT – Food Science and Technology, 65, 751–757. https://doi.org/10.1016/j.lwt.2015.08.069.
31. Savic, I. M., & Savic Gajic, I. M. (2025). Extraction and characterization of antioxidants and cellulose from green walnut husks. Foods, 14(3), 409. https://doi.org/10.3390/foods14030409.
32. Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids**.** *Biomedicine & Pharmacotherapy*, *56*(8), 365–379. https://doi.org/10.1016/S0753-3322(02)00253-6.
33. Stahlke, E. V. R., et al. (2019). Effects of Modified Atmosphere Packaging (MAP) and slaughter age on the shelf life of lamb meat. Food Science and Technology, 39(2), 328–335. https://doi.org/10.1590/fst.29617.
34. Steel, R. G. D., Torrie, J. H., & Dickey, D. A. Principles and procedures of statistics: A biometrical approach (3rd ed.). McGraw-Hill. (1997).
35. Tăpăloagă, D., Gheorghe-Irimia, R.A., Pogurschi, E.N., et al. (2023). Effects of Plant-Based Antioxidants in Animal Diets and Meat Products: A Review. *Foods*, *12*(6), 1334. https://doi.org/10.3390/foods12061334
36. Toomik, E., Rood, L., Bowman, J. P., & Kocharunchitt, C. (2023). Microbial spoilage mechanisms of vacuum-packed lamb meat: a review. *International Journal of Food Microbiology*, *387*, 110056. https://doi.org/10.1016/j.ijfoodmicro.2022.110056.
37. Vieira, V., Pereira, C., Pires, T. C. S. P., Calhelha, R. C., Alves, M. J., Ferreira, O., Barros, L., & Ferreira, I. C. F. R. (2019). Phenolic profile, antioxidant and antibacterial properties of Juglans regia L. (walnut) leaves from the Northeast of Portugal. Industrial Crops and Products, 134, 347–355. https://doi.org/10.1016/j.indcrop.2019.04.020.
38. Wang, L., Li, W., Li, X., Liu, J., & Chen, Y. (2023). Antimicrobial

ISSN 2072-3857

activity and mechanisms of walnut green husk extract. *Molecules, 28*(24), 7981. https://doi.org/10.3390/molecules28247981.

39. Yazdanparast, S., Mohammadi-Nasrabadi, F., Hashmati, A., Rezazadeh, R., Taheri, M., Alhouei, B., & Esfarjani, F. (2025). Comprehensive assessment of fatty acid profiles of meat products to develop action plan strategies for healthier products. *Scientific Reports, 15*(1), Article 1–11. https://doi.org/10.1038/s41598-025-04749-z.
40. Zhu, W., Han, M., Bu, Y., Li, X., Yi, S., Xu, Y., & Li, J. (2024). Plant polyphenols regulating myoglobin oxidation and color stability in red meat and certain fish: A review. *Critical Reviews in Food Science and Nutrition, 64*(8), 2276–2288. https://doi.org/10.1080/10408398.2022.2122922.

ISSN 2072-3857