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Research Article

Effects of adding pomegranate (*Punica granatum* L.) juice to marinade on the formation of malondialdehyde, glyoxal, and methylglyoxal in red meat

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ABSTRACT

This study investigated the impact of pomegranate juice (*Punica granatum* L.) as a marinade component on the formation of lipid oxidation- and glycation-related compounds in meat. Three different marination treatments (pomegranate juice, marinade, and a combination of both) and three marination durations (2, 6, and 24 hours) were applied. Malondialdehyde, glyoxal, and methylglyoxal levels were analysed via HPLC. The results revealed that pomegranate juice significantly reduced glyoxal formation, with the lowest glyoxal level recorded at 13.0 µg/100 g after 2 hours of marination ($p<0.001$). In contrast, 6-hour marination with traditional marinade resulted in the highest glyoxal level (174.6 µg/100 g). The malondialdehyde levels were lowest in the pomegranate juice group after 24 hours (109.3 µg/100 g), whereas the methylglyoxal levels peaked at 24 hours (468.3 µg/100 g). The combination of pomegranate juice and marinade had intermediate effects, reducing oxidation but maintaining moderate methylglyoxal. This study is limited by its focus on specific markers and the exclusion of sensory attributes and other potential oxidation markers. Factors such as meat type, fat content, and pH were not evaluated. Pomegranate juice shows promise as a natural antioxidant, but further studies should explore sensory evaluation, different meat types, and the long-term stability of antioxidant effects during storage to enhance practical applications.

Keywords: Glycation, Lipid oxidation, Marination, Pomegranate juice, Red meat

Introduction

Red meat plays a vital role in human nutrition because of its high nutritional content (Libera et al., 2021). However, the methods used in its preparation and consumption significantly impact both food safety and the sensory attributes of meat, such as flavour and aroma (Ashaolu et al., 2023). Grilling, one of the most popular cooking techniques for red meat, is particularly favoured alongside methods such as stewing and boiling (Li et al., 2023). Despite its widespread use, grilling and other high-temperature cooking methods are associated with the formation of mutagenic and hazardous compounds, which pose significant health risks, including an increased risk of cancer (Bukowska et al., 2023).

The thermal processing of meat, such as grilling, leads to the production of harmful compounds, including lipid oxidation products such as glyoxal (GO), methylglyoxal (MGO), and malondialdehyde (MDA), which negatively affect both food safety and human health (Yu et al., 2024). Lipid oxidation, a primary factor in meat quality degradation, not only alters flavour but also results in the production of harmful compounds associated with diseases such as cardiovascular disorders, cancer, and diabetes (Inan-Eroglu et al., 2020; Macho-González et al., 2021). Additionally, cooking processes contribute to the formation of advanced glycation end products (AGEs) through the Maillard reaction, particularly during high-temperature cooking methods such as frying and grilling (Inan-Eroglu et al., 2020). These AGEs are linked to chronic diseases, including cancer and diabetes, with GO and MGO serving as key precursors in their formation (Schalkwijk & Stehouwer, 2020; Wan et al., 2024).

To address these health risks, research has increasingly focused on natural antioxidants as potential solutions to mitigate the formation of harmful compounds during thermal processing. Polyphenols, which are abundant in various natural sources, have shown promise as effective antioxidants in meat and meat products (Domínguez et al., 2019; Papuc et al., 2017). For example, phenolic extracts from sources such as pomegranate, green tea, grape seeds, and rosemary have been found to reduce lipid and protein oxidation, whereas phenolic-rich marinades have been shown to mitigate cooking-induced chemical hazards (Dong et al., 2020; Gibis & Weiss, 2012; Keşkekoğlu & Üren, 2014).

In response to consumer demand for healthier and more natural food options, the meat industry is increasingly incorporating natural antioxidants such as polyphenols, flavonoids, and organic acids to replace synthetic additives (Hassanpour & Doroudi, 2023). Pomegranate, *Punica granatum* L. (*P. granatum*), with its high phenolic content and potent antioxidant properties, has emerged as a particularly promising natural preservative. Known for its health benefits, including antitumour and antioxidant effects, pomegranate holds significant potential for enhancing the nutritional value and safety of meat products (Singh et al., 2023).

Marination, a critical step in meat processing, enhances the tenderness, flavour, and shelf life of meat while also reducing the formation of harmful compounds during cooking (Ehsanur Rahman et al., 2023). Studies suggest that incorporating pomegranate juice into marinades can increase meat quality and reduce the formation of hazardous compounds, such as MDA, GO, and MGO, during cooking (Altun et al., 2024; Aydemir et al., 2024; Guo et al., 2021; Liu et al., 2014; Matthaiou et al., 2014; Yu et al., 2024).

Pomegranate juice, characterised by its rich profile of organic acids, phenolic compounds, and natural sugars, presents a multifaceted advantage as a marination component. Unlike conventional acidic marinades, it not only offers a balanced pH and antimicrobial efficacy but also contributes to the nutritional enhancement of meat products through its bioactive compounds. Moreover, its potent antioxidant capacity effectively mitigates lipid oxidation, thereby improving both the safety and sensory attributes of marinated meat (Gullón et al., 2020; Lytou et al., 2018). These multifaceted benefits supported its selection as a marinade component in this study.

This study aims to investigate the impact of adding pomegranate juice to meat marinades on the formation of MDA, GO, and MGO in red meat cooked at high temperatures. It is hypothesised that varying the duration of marination and the inclusion of pomegranate juice will significantly affect the levels of these harmful compounds, thereby offering a potential strategy for improving the health and safety of grilled meat.

Materials and Methods

Preparation of Samples

Pomegranates (P. granatum L.) were obtained from a local market in Gazipaşa, Antalya, and the pomegranate juices were squeezed by pressing. The beef tenderloin was obtained from local markets. The meat was sliced into equal thicknesses of 100 grams each, and 9 samples were obtained for different applications. Three different marinades (only pomegranate juice, only marinade, and marinade + pomegranate juice) and three different marinade times (2, 6, and 24 hours)

were applied. The application types and sample numbers are given in Table 1.

Marinating the Meat Samples

For the marination treatments, 30 mL of pomegranate juice or 30 mL of the standard marinade solution was applied individually to ensure a consistent liquid volume across all the samples. To evaluate the combined effects of pomegranate juice and the standard marinade, 30 mL of each solution (totalling 60 mL) was utilised, maintaining a fixed concentration of pomegranate juice across all experimental groups. The marination process involved complete immersion of the meat samples in the marinade, with intermittent mixing to promote uniform absorption. During subsequent analyses, only the marinade absorbed by the meat matrix was evaluated, excluding excess surface liquid. This approach ensured that the initial marinade volume did not influence the final analytical measurements, thereby providing reliable and comparable results across different treatment samples. The marinade solution contained 1.54% salt, 0.0068% black pepper, 0.0059% onion, 0.0146% coral mole, and 0.0008% rosemary (McKenna et al., 2003). One hund red grams of meat was marinated in 30 ml of marinade liquid for 2, 6, and 24 hours (M2, M6, and M24, respectively). One hundred grams of meat was mixed with 30 ml of pomegranate juice for 2, 6, or 24 hours (P2, P6, P24). One hundred grams of meat was marinated in 30 ml of pomegranate juice + 30 ml of marinade mixture for 2, 6, or 24 hours (PM2, PM6, or PM24). The meat was marinated via the immersion method. To ensure consistency across all samples, the marination solutions were prepared in standardised volumes and concentrations. Each sample was fully submerged in the designated marinade, with consistent mixing applied to maintain uniform exposure to the marination solution. The marinating process was conducted at a controlled temperature below 4.4°C to prevent microbial growth and ensure sample stability (Smith & Acton, 2000).

Cooking the Meat Samples

Following the marination period, the meat was cooked on an electric grill at 230°C, a temperature commonly used in household grilling practices. The cooking process was continued until the internal temperature of the meat reached 75°C, which aligns with the recommended safe cooking temperature for red meat according to food safety guidelines. The internal temperature of the meat was monitored and regulated via a probe thermometer. The cooked meats were then packaged separately and labelled with the corresponding sample number. The prepared samples were vacuum-packed to prevent contact with oxygen and stored at -18°C until analysis.

Chemical Analysis

AGE Precursors (Glyoxal and Methylglyoxal)

Analyses of glyoxal (GO) and methylglyoxal (MGO) in the samples were performed using an HPLC system after extraction and derivatisation procedures. A 0.1 N hydrochloric acid solution was prepared by mixing 8.28 mL of concentrated hydrochloric acid with deionised water to a final volume of 1000 mL. The mobile phase for HPLC consisted of methanol, water, and acetonitrile at a 42:56:2 (v/v/v) ratio.

For extraction and derivatisation, 5 g of each sample was homogenised in 25 mL of methanol using an Ultra-Turrax homogeniser for 1 minute, followed by centrifugation at 8000 rpm for 5 minutes. A 0.5 mL aliquot of the supernatant was transferred into a glass tube and mixed with phosphate buffer (pH 3) and 0.5 mL of a derivatisation solution containing 4-nitro-1,2-phenylenediamine (50 mg dissolved in 50 mL of methanol). The mixture was incubated at 70°C in a water bath for 30 minutes. After derivatisation, the solution was filtered through a 0.45 µm cellulose acetate filter and injected into the HPLC system.

HPLC analysis was carried out using an Inertsil Zorbax C-18 column (4.6 mm × 150 mm). The detection wavelength was set to 255 nm, with an injection volume of 10 µL and a flow rate of 1 mL/min. This method ensures efficient separation and quantification of GO and MGO, enabling the assessment of AGE precursors in the analysed samples.

Table 1. Sample Groups based on Marination Type and Duration

Marinating solutions	Marination duration		
	2 h	**6 h**	**24 h**
Pomegranate juice (30 mL)	P2	P6	P24
Marinade (30 mL)	M2	M6	M24
Pomegranate juice (30 mL) + Marinade (30 mL)	PM2	PM6	PM24

Malondialdehyde (MDA)

The determination of MDA was carried out via HPLC following the preparation of derivatised samples and the mobile phase. A trichloroacetic acid (TCAA) solution (10%) was prepared by dissolving 100 g of TCAA in deionised water to a final volume of 1000 mL. Thiobarbituric acid (TBA) solution (0.67%) was prepared by dissolving 1.675 g of TBA in double-distilled water and diluting it to 250 mL. A tetraethoxypropane standard was prepared by diluting 0.5 mL of the stock solution in 100 mL of ethanol and further diluting 0.1 mL of this solution to 100 mL with deionised water to achieve a final concentration of 20 µL/L. For the pregallol solution (7.2%), 7.2 g of pregallol was dissolved in 100 mL of ethanol. For standard preparation, 0.1 mL of tetraethoxypropane solution was added to a test tube, followed by the addition of 1 mL of TCAA and 1 mL of TBA solution. The mixture was incubated in a water bath at 90°C for 10 minutes, cooled, filtered through a 0.45 µm cellulose acetate filter, and analysed via HPLC. The mobile phase was prepared by mixing 0.05 M KH_2PO_4 buffer solution with methanol and acetonitrile at a 72:17:11 (v/v/v) ratio.

The samples (5 g each) were homogenised in 25 mL of TCAA solution using an Ultra-Turrax homogeniser for 1 minute and then centrifuged at 8000 rpm for 5 minutes. A 1 mL aliquot of the supernatant was mixed with 1 mL of TBA solution for derivatisation and incubated in a water bath at 90°C for 30 minutes. The derivatised samples were filtered through a 0.45 µm cellulose acetate filter and analysed via HPLC.

HPLC analysis was performed via a Shimadzu HPLC-UV system equipped with an Inertsil ODS-3 column (4.6 mm × 150 mm). The wavelength was set to 530–550 nm, the injection volume was 10 µL, and the flow rate was 1 mL/min. This method allowed for the precise quantification of MDA in the samples.

Statistical Analyses

All the analyses were carried out in triplicate, and the data are presented as the means ± SD. For all the data, univariate analysis of variance (ANOVA) with Tukey's post hoc test was applied via the SPSS 26.0 package program. Statistical significance was accepted as $p < 0.05$ in all analyses.

Results and Discussion

As shown in Table 2, the glyoxal levels in grilled beef tenderloins varied significantly based on the marination type and duration. Pomegranate juice alone resulted in the lowest glyoxal levels, with 2-hour marination yielding 13.0 µg, which was significantly lower than that at 6 hours (171.6 µg) and 24 hours (93.3 µg) ($p<0.05$). In contrast, marination with marinade alone showed no clear time-dependent trend, with levels increasing from 60.3 µg at 2 hours to 174.6 µg at 6 hours and decreasing to 109.3 µg at 24 hours ($p<0.05$). The combination of pomegranate juice and marinade had the lowest glyoxal level at 24 hours (72.3 µg), which was significantly lower than that at 2 hours (143.3 µg) and 6 hours (133.6 µg) ($p<0.05$). Across all the conditions, the lowest glyoxal level was observed with 2-hour pomegranate juice marination (13.0 µg), and the highest glyoxal level was observed with 6-hour marination-only marination (174.6 µg).

Cooking meat can produce harmful compounds such as glyoxal, but recent studies have shown that natural ingredients, such as herbs and polyphenol-rich extracts, can significantly reduce their formation (Altun et al., 2024; Aydemir et al., 2024; Liu et al., 2014; Yu et al., 2024). In one study, beef tenderloins were marinated in cranberry juice at two different concentrations (25% and 50%) for three durations (2, 6, and 24 hours) and then cooked in an air fryer at 200°C for 12 minutes. The results revealed that increasing the cranberry juice concentration and marination time significantly inhibited the formation of the AGE precursor Nε-(carboxymethyl)lysine (CML), whereas Nε-(carboxyethyl)lysine (CEL) formation remained unaffected. The CML level decreased by up to 12.39 µg/g, reaching as low as 1.2 µg/g (Altun et al., 2024). Similarly, in Aydemir et al.'s (2024) study, beef tenderloins were marinated in hawthorn vinegar at two different concentrations (25% and 50%) and for three different durations (2, 6, and 24 hours), followed by cooking in an air fryer at 200°C for 12 minutes. As a result, increasing the concentration and marination time significantly inhibited the formation of CML and CEL. The 24-hour marination process reduced CML levels from 13.75 µg/g to 2.5 µg/g, whereas CEL levels decreased from 17.58 µg/g to 16.63 µg/g. Although CEL formation was inhibited at low levels, it remained stable (Aydemir et al., 2024). In another study, beef patties were divided into four groups: a control group without spices and three experimental groups containing 0.5% (w/w) rosemary, turmeric, or bay leaf powder. The patties were mixed at 4°C for 12 hours and then heated at 230°C for 15 minutes in an oven. The addition of 0.5% rosemary, turmeric, or bay leaf to roasted beef patties significantly reduced glyoxal levels, with inhibition rates of 49.07% for rosemary, 14.72% for turmeric, and 7.89% for bay leaf (Yu et al., 2024). The glyoxal inhibition observed with pomegranate juice in our study could be attributed to its specific polyphenolic profile shown in previous studies (Altun et al., 2024; Aydemir et al., 2024; Liu et al., 2014). Air-fried beef tenderloins marinated with concentrated juice reported that compounds such as quinic acid, gallic acid, protocatechuic acid, and ellagic acid, which

are found in high amounts in the juice, inhibit the formation of AGEs by binding to glyoxal (Altun et al., 2024; Aydemir et al., 2024) Furthermore, several compounds, including quercetin (Liu et al., 2014), catechin, epigallocatechin, and kaempferol (Aydemir et al., 2024), which highly contain pomegranate, have been identified as inhibitors of AGE formation in foods. These findings align with our results, suggesting that the rich polyphenolic content of pomegranate juice plays a critical role in mitigating glyoxal formation and subsequent AGE development during cooking processes.

Our findings revealed that marinating meat in pomegranate juice for 2 hours resulted in the lowest levels of glyoxal. In contrast, marination periods of 6 or 24 hours led to increased glyoxal formation. Short-term marination (20 minutes to 3 hours) has been shown to improve water retention, enhance texture, and reduce the formation of harmful compounds in cooked meat (Goli et al., 2014). However, longer marination durations (6–24 hours) are not considered to provide additional benefits, as they may lead to adverse effects, including a reduction in antioxidant properties and an increase in protein degradation (Kęska et al., 2019).

The methylglyoxal (MGO) levels in grilled beef tenderloins varied based on the marination type and duration (Table 3). For samples marinated with pomegranate juice alone, the highest MGO level was observed after 24 hours (468.3 μg), which was significantly greater than the level after 2 hours (287 μg) ($p<0.05$). In contrast, for samples marinated with marination alone, there were no significant differences in MGO levels across marination durations ($p>0.05$).

When marinated with a combination of pomegranate juice and marinade, the highest MGO level was observed at 6 hours (401.3 μg), and the lowest at 24 hours (324 μg), with a significant difference between these times ($p < 0.05$). At 2 h, the highest MGO level was detected in the combination group (382.3 μg), whereas the lowest was detected in the marinade-only group (195.6 μg) ($p<0.05$).

After 24 hours, the highest MGO level was recorded in the pomegranate juice group (468.3 μg), and the lowest was recorded in the marinade-only group (236 μg) ($p < 0.05$). These results emphasise that marination type and duration significantly influence MGO levels, with pomegranate juice contributing to higher levels over extended marination periods.

In meat products, methylglyoxal (MGO) can form during processing and cooking, particularly during grilling (Duman & Kurban, 2022). Our findings partially align with the literature, which demonstrates that certain polyphenolic compounds in pomegranate juice, such as punicalagin, ellagic acid, and gallic acid, have a strong ability to inhibit nonenzymatic glycation by scavenging MGO (Liu et al., 2014). Specifically, punicalagin exhibited vigorous antiglycation activity, reducing the MGO content by 84.6% (Liu et al., 2014). Additionally, Yu et al. (2024) reported that natural plant-based antioxidants such as rosemary, turmeric, and bay leaf reduced MGO levels in meat products by 41.81%, 21.30%, and 13.93%, respectively. However, our results indicate that prolonged marination with pomegranate juice alone may lead to increased MGO levels, potentially due to the extended exposure of meat to sugars and reactive carbonyl species within the juice (Duman & Kurban, 2022). Furthermore, studies on dietary supplementation with *P. granatum* L. concentrate have demonstrated a 9.8% reduction in plasma MGO levels after 4 weeks (Bednarska et al., 2023), revealing the antiglycation effects of pomegranate-derived compounds.

Marination with polyphenol-rich extracts can reduce lipid oxidation in meats; however, prolonged marination may not always be beneficial, as it can increase protein and peptide hydrolysis, diminishing antioxidant properties (Kęska et al., 2019). In our study, prolonged marination resulted in browning until the internal temperature reached 75°C, likely due to changes in the pomegranate juice composition over time. Its high organic acid content, including ellagic acid, ascorbic acid, malic acid, and citric acid, along with carboxylate groups, may contribute to this effect (Renda & Şöhretoğlu, 2024). Weak acids and their conjugate bases catalyse sugar anomerization and degradation, whereas carboxylate ions promote Maillard and other nonenzymatic browning reactions by generating reactive intermediates from sugars (Kaufmann et al., 2018). These reactions, along with acid-induced protein modifications, may explain the increase in MGO levels observed with prolonged marination.

The malondialdehyde (MDA) levels also varied significantly depending on the type and duration of marination (Table 4). For samples marinated with pomegranate juice alone, the lowest MDA level was observed after 24 hours (109.3 μg), which was significantly lower than that observed after 2 hours (221.6 μg) or 6 hours (196.6 μg) ($p<0.05$). No significant differences were noted between 2 and 6 hours ($p>0.05$).

For samples marinated with the marinade alone, no significant differences in MDA levels were found across marination durations ($p>0.05$). In contrast, for samples marinated with a combination of pomegranate juice and marinade, the MDA levels decreased with increasing marination time, from 360.3 μg at 2 hours to 141.3 μg at 24 hours ($p<0.05$).

Across all marination conditions and durations, the highest MDA level was observed after 2 hours of marination with

pomegranate juice + marinade (360.3 µg). In contrast, the lowest was found after 24 hours of marination with pomegranate juice alone (109.3 µg) ($p<0.05$). For 24-hour marination, the MDA levels were significantly different between the marination types, with the highest level in the marination-only group (183.6 µg) and the lowest in the pomegranate juice group (109.3 µg) ($p<0.05$).

These results align with findings in the literature, which report the antioxidative effects of pomegranate-derived products on lipid oxidation. Yu et al. (2024) demonstrated that spices such as rosemary and turmeric significantly reduce MDA levels, resulting in inhibition rates of up to 93.46%. Similarly, Turgut et al. (2017) and Naveena et al. 2008 reported that pomegranate peel extract and juice effectively inhibited MDA formation in beef meatballs and cooked chicken patties during storage.

Table 2. Effects of different marinating solutions and times on the GO content

Samples	Mean ±SD (µg/100 g)	95% CI	*p* value
P2	13.0 ±1.0^{a}	10.5 - 15.4	
M2	60.3 ±2.5^{b}	54.1 - 66.5	
PM2	143.3 ±6.5^{c}	127.1 - 159.4	
P6	171.6 ±8.0^{d}	151.7 - 191.5	
M6	174.6 ±8.0^{d}	154.7 - 194.5	<0.001
PM6	133.6 ±6.0^{c}	118.6 - 148.6	
P24	93.3 ±4.5^{e}	82.1 - 104.5	
M24	109.3 ±4.5^{f}	98.1 - 120.5	
PM24	72.3 ±3.5^{b}	63.6 - 81.1	

Different superscript letters (a-f) within the same column indicate statistically significant differences between groups ($p < 0.05$) as determined by one-way ANOVA and Tukey's post hoc test. Identical letters denote no significant difference between values

Table 3. Effect of different marinating solutions and times on MGO levels

Samples	Mean ±SD (µg/100 g)	95% CI	*p* value
P2	287.0 ±12.5^{a}	255.8 - 318.1	
M2	195.6 ±9.0^{b}	173.2 - 218.1	
PM2	382.3 ±17.0^{c}	340.0 - 424.6	
P6	369.0 ±16.5^{c}	327.9 - 410.0	
M6	201.6 ±9.0^{b}	179.2 - 224.1	<0.001
PM6	401.3 ±18.0^{c}	356.5 - 446.1	
P24	468.3 ±21.0^{d}	416.1 - 520.5	
M24	236.0 ±10.5^{b}	209.8 - 262.1	
PM24	324.0 ±14.5^{a}	287.9 - 360.1	

Different superscript letters (a-d) within the same column indicate statistically significant differences between groups ($p < 0.05$) as determined by one-way ANOVA and Tukey's post hoc test. Identical letters denote no significant difference between values

Pomegranate juice likely prevents MDA and glyoxal formation through multiple mechanisms. Rich with polyphenols such as ellagic acid, punicalagin, and anthocyanins, it neutralises reactive carbonyl species and inhibits lipid peroxidation (Liu et al., 2014; Matthaiou et al., 2014). Additionally, its consumption increases glutathione (GSH) levels. It enhances the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST), while reducing MDA levels (El-Shehawi et al., 2022; Shaban et al., 2014). Pomegranate juice also modulates key signalling pathways, such as the NF-κB and NRF2 pathways, to mitigate inflammation, contributing to its protective role against acrylamide-induced oxidative stress (El-Shehawi et al., 2022; Mukherjee et al., 2021). Furthermore, punicalagin reduces glyoxal accumulation, inhibits the formation of AGEs, and accelerates glyoxal metabolism and excretion (Liu et al., 2014).

Our findings further support the potential of pomegranate juice as a natural antioxidant in meat products, particularly when used as a marinade. In vivo research has shown that consuming pomegranate juice or pomegranate extract with a high-fat hamburger pattern can reduce postprandial urinary MDA levels in humans (Henning et al., 2017). Additionally, pomegranate rind extract is an effective alternative to synthetic preservatives in fat-rich meat products, improving lipid oxidative stability and microbiological characteristics during refrigerated storage (Dua et al., 2016). Our findings similarly show that pomegranate juice can improve the oxidative stability of grilled beef tenderloins, with the lowest MDA levels observed after 24 hours of marination.

The primary aim in cooked meats is to minimise hazardous compounds (Yu et al., 2024), with the addition of antioxidants being a key strategy to inhibit lipid oxidation in meat products (Domínguez et al., 2019). Polyphenols are effective antioxidants in meat products because of their ability to chelate prooxidative metals, scavenge free radicals, and control lipid oxidation (Nikmaram et al., 2018; Papuc et al., 2017). Studies on culinary spices and herbs have shown that their effectiveness in limiting lipid oxidation during the cooking of high-fat beef and beef meatballs is strongly correlated with their phenolic content (Keşkekoğlu & Üren, 2014; Liu et al., 2014; Matthaiou et al., 2014; Xie et al., 2022). Studies have shown that polyphenols in marinades significantly reduce lipid oxidation, whereas the marination process enhances polyphenol distribution, maximising antioxidant effects (Keşkekoğlu & Üren, 2014; Xie et al., 2022). In one study, beef and chicken patties containing 0.5% (w/w) pomegranate seed extract were cooked via four different methods: baking, pan-frying, grilling, and deep-frying. Pomegranate seed extract reduced total heterocyclic aromatic amine (HAA) formation by 39% during grilling and 46% during deep-frying in beef patties. In contrast, in chicken patties, it decreased HAA formation by 49% during deep-frying. However, a 70% increase in HAA formation was observed during baking (Keşkekoğlu & Üren, 2014). Another study evaluated the impact of tea polyphenol (TP, 0.3 g/kg) application on lipid oxidation by dividing the samples into three groups, all of which were stored at 4°C for 2 hours: the control group was stored directly, the unmarinated group was first stored and then treated with TP, and the marinated group was first treated with TP and then stored. The results demonstrated that the marinated samples presented the lowest peroxide value, TBARS level, polyunsaturated fatty acid loss, free radical level, and lipoxygenase activity, suggesting that the marination process enhanced the antioxidant effects of tea polyphenols, thereby effectively inhibiting lipid oxidation (Xie et al., 2022). Pomegranate juice is an abundant source of polyphenolic compounds that can effectively reduce lipid oxidation (Liu et al., 2014; Matthaiou et al., 2014).

This study has several limitations. The study focused solely on beef tenderloin, which limits the generalizability of the findings to other meat types. The study did not assess how factors such as meat type, fat content, and pH might influence oxidation and glycation reactions. Additionally, only grilling at 230°C was evaluated, and other cooking methods may yield different outcomes. The analysis was limited to specific oxidative markers (GO, MGO, and MDA), excluding other potential markers such as hydroperoxides. Furthermore, the study did not assess sensory attributes such as taste and texture, which could impact the practical applicability of the findings. Despite these limitations, this study provides new insights into the antioxidant potential of pomegranate juice in meat marination. The experimental design included multiple marination durations (2, 6, and 24 hours) to evaluate the time-dependent effects. The use of HPLC analysis enhances data reliability. These findings support the use of natural antioxidants as alternatives for reducing harmful oxidation products in grilled meats, with potential benefits for the food industry.

Table 4. Effect of different marinating solutions and times on the MDA content

Samples	Mean±SD (µg/100 g)	95% CI	*p* value
P2	221.6 ±10.0^{d,e}	196.7 - 246.5	
M2	198.6 ±9.0^{c,d}	176.2 - 221.1	
PM2	360.3 ±16.0^{f}	320.4 - 400.1	
P6	196.6 ±9.0^{c,d}	174.2 - 219.1	
M6	209.6 ±9.0^{c,d,e}	187.2 - 232.1	<0.001
PM6	233.0 ±10.5^{e}	206.8 - 259.1	
P24	109.3 ±4.5^{a}	98.1 - 120.5	
M24	183.6 ±8.0^{c}	163.7 - 203.5	
PM24	141.3 ±6.5^{b}	125.1 - 157.4	

Different superscript letters (a-f) within the same column indicate statistically significant differences between groups ($p < 0.05$) as determined by one-way ANOVA and Tukey's post hoc test. Identical letters denote no significant difference between values

Conclusion

This study highlights the potential of pomegranate juice as a natural marinade to mitigate oxidation and glycation-related compounds in grilled beef. These results demonstrate that pomegranate juice effectively reduces glyoxal and malondialdehyde levels, enhancing the nutritional quality and safety of cooked meat. However, its impact on methylglyoxal formation requires further investigation.

Given the increasing consumer demand for natural food preservatives, incorporating pomegranate juice into meat processing may offer a practical and health-promoting alternative to synthetic additives. Future research should focus on optimising marination conditions, assessing the effects of marination on different meat types, and evaluating sensory attributes to improve consumer acceptance. Additionally, studies exploring the long-term stability of antioxidant effects during storage and the impact of pomegranate juice on the sensory qualities of meat products are recommended. These findings contribute to the broader application of natural antioxidants in the food industry, supporting efforts to develop healthier and safer meat products.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare that they have no actual, potential, or perceived conflicts of interest related to this article.

Ethics committee approval: The authors declare that this study does not involve experiments with human or animal subjects, and therefore, ethics committee approval is not required.

Data availability: Data will be made available at the request of the author(s).

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