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Effects of beverages and salads on lipid oxidation and glycation during in vitro meat digestion

Güleren Sabuncular[1]✉, Gamze Akbulut[2] & Mustafa Yaman[3]✉

This study investigates the impact of various beverages and salads on lipid oxidation and the formation of malondialdehyde (MDA), glyoxal (GO), and methylglyoxal (MGO) during in vitro gastrointestinal digestion of beef and chicken. Meat samples were digested alongside beverages (grape juice, red wine, pomegranate juice, ayran, hardaliye, and shalgam) and salads (Mediterranean, red cabbage, and coban salad). HPLC analysis revealed that red wine, pomegranate juice, hardaliye, and shalgam significantly reduced MDA levels in both meat types (up to 77%), while grape juice increased MDA in beef (27%) and orange juice in chicken (80%). Shalgam was most effective in reducing MGO levels (54%), though red wine, pomegranate juice and, hardaliye increased GO and MGO levels. Red cabbage and Mediterranean salads reduced MDA across all samples, while coban salad was less effective. Ayran reduced MDA in both meats but increased MGO, especially in chicken (102.9%). Results indicate that antioxidant-rich beverages and salads can mitigate lipid oxidation and oxidative stress markers in meats during digestion, varying effectiveness by component. This highlights the value of selecting dietary additions to enhance meat quality and reduce oxidative damage.

Keywords Glyoxal, In vitro gastrointestinal digestion, Lipid oxidation, Malondialdehyde, Methylglyoxal

Oxidative stress, a condition characterized by an imbalance between reactive oxygen species and antioxidant defenses, plays a critical role in the deterioration of food quality, particularly in meat products. The digestive system is a significant entry point for oxidized substances made during meat processing and storage. However, lipid oxidation can worsen during digestion[1]. Under gastrointestinal digestion conditions, the gastric environment functions as a bioreactor that promotes lipid oxidation; this process is accelerated by the presence of reactive components such as emulsified lipids, iron, and oxygen, ultimately leading to the formation of harmful oxidative products[2]. Some of these oxidative products are malondialdehyde (MDA), glyoxal (GO), and methylglyoxal (MGO), which are precursors of advanced glycation end products (AGEs)[3].

Previous studies have reported significant increases in MDA levels in beef and chicken meat following digestion, highlighting their susceptibility to these meats to oxidative damage[4–6]. For instance, Van Hecke et al.[4], tested the potential of ten culinary herbs and spices to limit oxidation during the cooking and in vitro digestion of a high-fat beef product, reporting that sweet paprika exerted an antioxidant effect during digestion, whereas garlic did not[4]. Han et al.[5] demonstrated that olive oil polyphenols significantly inhibited lipid oxidation during in vitro digestion of high-fat beef, with compounds such as 3,4-DHPEA-EDA and hydroxytyrosol exhibiting phase-specific antioxidant activity in the gastric and intestinal stages, respectively[5]. However, most studies have focused on individual antioxidants or isolated compounds rather than whole food matrices such as beverages and salads, which may have synergistic effects.

The composition of meat, particularly its high levels of polyunsaturated fatty acids and iron content, further exacerbates oxidation during digestion[7]. Therefore, strategies to mitigate oxidative damage in meat products are essential for maintaining their quality and nutritional value.

One promising approach to combating oxidative stress in meat products involves the incorporation of antioxidant-rich fruits and vegetables. Grapes, for instance, are abundant in phenolic compounds such as anthocyanins, resveratrol, and quercetin, which are known for their antioxidant properties[8]. Human studies have shown that grape juice can reduce oxidative stress markers, including MDA[9,10]. Similarly, red wine, which contains higher levels of bioactive compounds than grape juice, has been documented to inhibit lipid oxidation

[1]Department of Nutrition and Dietetics, Faculty of Health Sciences, Marmara University, Başıbüyük Health Campus, No: 3 34854 Başıbüyük, Maltepe, Istanbul, Türkiye. [2]Department of Nutrition and Dietetics, Faculty of Health Sciences, İstanbul Kent University, Istanbul, Türkiye. [3]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Istanbul Sabahattin Zaim University, Küçükçekmece, Istanbul, Türkiye. ✉email: gulerenserin@gmail.com; mustafa.yaman@izu.edu.tr

in meat[11,12]. Pomegranate, rich in flavonoids and exhibiting strong antioxidant properties, has been found to possess three times the antioxidant activity of red wine[13]. Furthermore, traditional fermented beverages like hardaliye and shalgam, made from red grapes and black carrots, are also recognized for their high antioxidant potential[14,15].

Vegetables, akin to fruits, are rich in antioxidants and can help prevent oxidation in meat products[16]. Specific vegetables like red cabbage have effectively reduced lipid oxidation due to their stable anthocyanin content[17]. In vitro studies have identified spinach, broccoli, and red peppers as effective at inhibiting lipid oxidation in turkey meat, while tomatoes and green peppers exhibited lower antioxidant activity[18]. Salads, particularly those incorporating Mediterranean ingredients, have shown promise in reducing MDA formation during digestion[18,19].

While previous studies have investigated the impact of individual polyphenols or extracts on lipid oxidation, the present study expands on this by exploring the effects of whole food matrices—specifically antioxidant-rich beverages and salads—on lipid oxidation in both beef and chicken during in vitro gastrointestinal digestion. By evaluating these dietary components within a more realistic consumption model, this study seeks to provide additional insights into potential dietary strategies for mitigating oxidation in meat products and enhancing overall meal quality.

Materials and methods

Chemicals and enzymes

All chemicals and reagents used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). The enzymes used included lipase (100–500 U/mg protein), alpha-amylase (1.5 U/mg), pepsin (≥250 units/mg solid), pancreatin (8×USP), and mucin. The salts and buffers utilized in the experiments comprised NaCl, KCl, $CaCl_2 \cdot 2\ H_2O$, $NaHCO_2$, and urea. Bovine serum albumin (BSA) was used as a protein standard. The solvents and reagents included glyoxal (40%), methylglyoxal (40%), methanol, acetonitrile (ACN), sodium acetate, 4-nitro-1,2-phenylenediamine, meta-phosphoric acid, thiobarbituric acid, trichloroacetic acid (TCA), and bile salts mixture.

Preparation of samples

Beef round and chicken breast meat were used in the study. Meats were purchased from a local supermarket in Türkiye. Seven types of beverages (freshly squeezed orange juice, 100% grape juice, red wine, hardaliye (a fermented grape drink), shalgam (fermented black carrot juice), freshly squeezed pomegranate juice, and ayran) and three types of salads (Mediterranean salad, coban salad, and red cabbage salad) were used for co-digestion with the meat. Salads, orange juice, and pomegranate juice were freshly prepared on the analysis day, while other beverages were purchased from a local supermarket in Türkiye. Mediterranean salad contained 50 g of carrot, 50 g of iceberg lettuce, 10 g of corn, 30 g of tomato, 2 g of fresh basil, juice of 50 g lemon, 5 g of vinegar, 5 g of extra virgin olive oil, and 0.5 g of salt. Coban salad contained 30 g of green pepper, 50 g of cucumber, 20 g of onion, 150 g of tomato, 2 g of fresh basil, 2 g of fresh mint, juice of 50 g lemon, 5 g of extra virgin olive oil, and 0.5 g of salt. Red cabbage salad contained 50 g of carrot, 200 g of red cabbage, 2 g of fresh basil, juice of 50 g lemon, 10 g of vinegar, 5 g of extra virgin olive oil, and 0.5 g of salt. The meats were cooked on an electric grill (Kenwood HG230, 2200 W, Kenwood, UK) until the internal temperature reached 75 °C, then blended using a blender (Philips, Netherlands) and vacuum packed using a vacuum sealer (Vestel, Türkiye) to prevent exposure to oxygen. Samples were stored at − 20 °C until analysed. The amounts of meat and beverages used for in vitro digestion were determined based on standard portion sizes outlined in the National Nutrition Guide of Türkiye to ensure physiological relevance. One portion of meat (80 g) was prepared for co-digestion with one portion of beverage (200 mL) or one portion of salad (150 g). The final sample mixtures consisted of 5 g of meat combined with either 12.5 mL of beverage or 9.4 g of salad, ensuring consistency across all experimental groups.

To ensure the freshness of the meat samples, their delivery date was inquired at the supermarket and selected the freshest available products. All meat samples used in the study were sourced from the same batch to maintain consistency. Following the cooking process, the samples were homogenized through blending, ensuring uniform composition and standardized storage and processing conditions across all experimental groups.

In vitro digestion

The in vitro gastrointestinal digestion was performed according to the INFOGEST® 2.0 protocol[20], with modifications to the specific conditions proposed by Lee et al.[21]. The preparation of mouth, stomach, small intestine, and bile solutions were shown in Fig. 1.

In this study, 5 g of each meat sample was co-digested with 12.5 mL of beverages and 9.4 g of salads. The samples were homogenized using a homogenizer (IKA® T18 Digital Ultra Turrax, IKA, Germany) with 5 mL of saliva juice and incubated for 5 min at 37 °C in a shaking water bath (Memmert, Germany). Subsequently, 12 mL of gastric juice solution was introduced, and the samples were incubated for 30 min at 37 °C in the same conditions. To simulate intestinal conditions, 5 mL of bile solution and 10 mL of duodenal juice were added, the pH was adjusted to 7, and the mixture was incubated for 2 h at 37 °C. The reaction was then halted by adding 10% TCA, and the volume was adjusted to 50 mL using deionized water. The samples were centrifuged at 10,000 rpm for 10 min at 22 °C using a centrifuge (Himac CR22N, Hitachi, Japan), filtered through a 0.45 µm cellulose acetate filter, and then injected into the HPLC system for analysis.

Malondialdehyde analysis

MDA determination was based on the modified methods of Bertolín et al.[22], and Zhang et al.[23], with tetraethoxypropane used to prepare the MDA standard. A stock solution was prepared by adding 0.5 mL of tetraethoxypropane to a 100 mL volumetric flask and diluting with ethanol, with each standard completed using 10% TCA. For each meat sample, 30 mL of 10% TCA solution was added to 50 mL falcon tubes, and the samples

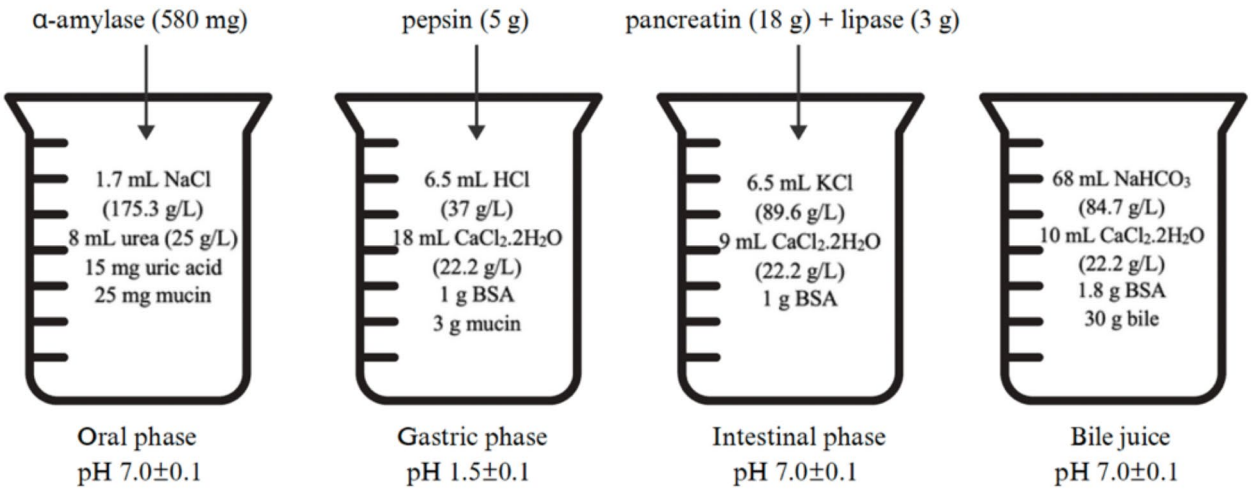

Fig. 1. In vitro human digestion system procedure.

were homogenized, at 13,000 rpm for 1 min at 4 °C using a homogenizer (IKA® T18 Digital Ultra Turrax, IKA, Germany), followed by centrifugation at 10,000 rpm for 2 min at 22 °C using a centrifuge (Himac CR22N, Hitachi, Japan). The volume was adjusted to 50 mL with TCA, and a second centrifugation was performed at 15,000 rpm for 10 min at 22 °C. Then, 1 mL of the supernatant was reacted with 1 mL of 0.67% thiobarbituric acid solution, heated at 90 °C for 30 min, cooled at room temperature, filtered through a 0.45 µm cellulose acetate filter, and injected into the HPLC system.

Glyoxal and methylglyoxal analysis

Determination of GO and MGO was based on the modified methods of Cengiz et al.[24]. The sample was placed in a 50 mL falcon tube and 25 mL of methanol was added. The sample was homogenized for 1 min at 4 °C using an ultra-thorax homogenizer (IKA® T18 Digital Ultra Turrax, IKA, Germany) and then centrifuged at 8000 rpm for 5 min at 22 °C using a centrifuge (Himac CR22N, Hitachi, Japan). 0.5 mL of the centrifuged supernatant was transferred to a glass tube and phosphate buffer (pH = 3) was added. Then 0.5 mL of 4-nitro-1,2-phenylenediamine solution (50 mg/50 mL methanol) was added for derivatisation and incubated in a water bath at 70 °C for 30 min. It was then filtered through a 0.45 µm cellulose acetate filter and injected into the HPLC.

HPLC parameters

The HPLC was consisted of a Shimadzu LC-20AT pump coupled with a Shimadzu SPD-20 A UV/VIS detector (Shimadzu Corporation, Kyoto, Japan). The separation was performed on an Inersil ODS-3 C18 column (4.6 mm × 250 mm, 5 µm particle size) at a column temperature of 30 °C. The mobile phase was composed of 0.05 M KH_2PO_4 buffer solution/methanol/acetonitrile (72/17/11, v/v/v), and the flow rate was maintained at 1 mL/min. A fluorescence detector was used with an excitation wavelength of 530 nm and an emission wavelength of 550 nm. The injection volume was 10 µL, and the system operated under an isocratic elution mode with a total run time of 15 min.

Calculation of inhibition and promotion

The percent MDA, GO, MGO inhibition by salads and beverages was calculated using Tang et al.'s formula[25].

$$\text{Increase decrease } \% = (\text{after digestion} - \text{before digestion})/\text{before digestion} \times 100.$$

$$\text{Inhibition or promotion} = (\text{after digest with salads} - \text{after digestion meat only})/\text{after digestion meat only} \times 100.$$

Statistical analysis

All analyses were performed in triplicate, with data presented as mean ± SD. ANOVA with Tukey's post-hoc test (Minitab v15) was used for multiple comparisons, considering $p < 0.05$ as significant.

Results

Table 1 shows the MDA levels before and after digestion of beef round and chicken breast samples. In the beef round, MDA levels increased significantly after digestion, rising from 268.7 ± 12.2 µg to 1357.1 ± 61.4 µg, a 405.1% increase. Co-digestion with beverages like grape juice, ayran, red wine, hardaliye, and shalgam altered MDA levels compared to beef round alone, with grape juice reducing MDA by 36.4% pre-digestion but still resulting in higher post-digestion levels. Significant reductions were observed with pomegranate juice (75.9%), shalgam (61.2%), red wine (53.6%), hardaliye (48.4%), and ayran (44.4%) ($p < 0.05$). Grape juice increased MDA by 27.0%. Similarly, in chicken breast, MDA levels rose from 91.7 ± 4.2 µg to 669.8 ± 30.3 µg, a 630.4% increase. Co-digestion with all beverages and salads, except ayran, resulted in lower post-digestion MDA levels than pre-

Samples	MDA (µg)/80 g meat + 150 g salad or 200 mL beverage		Increase/decrease and inhibition % after digestion	
	Before digestion (mean ± SD)	After digestion (mean ± SD)	Increase/decrease	Inhibition
Beef round	268.7 ± 12.2	1357.1 ± 61.4^{B,C}	405.1	–
Beef round + Grape juice	2710.5 ± 122.6	1723.0 ± 78.0^{A}	− 36.4	− 27.0
Beef round + Orange juice	4030.1 ± 182.4	1251.0 ± 56.6^{C}	− 69.0	7.8
Beef round + Red wine	472.1 ± 21.4	629.1 ± 28.5^{E,F}	33.3	53.6
Beef round + Hardaliye	4664.1 ± 211.0	700.9 ± 31.7^{E}	− 85.0	48.4
Beef round + Shalgam	346.5 ± 15.8	527.0 ± 23.8^{F}	52.1	61.2
Beef round + Pomegranate juice	1743.7 ± 79.0	327.7 ± 14.8^{G}	− 81.2	75.9
Beef round + Ayran	324.5 ± 14.8	754.3 ± 34.1^{E}	132.4	44.4
Beef round + Coban salad	905.6 ± 41.0	1427.2 ± 64.6^{B}	57.6	− 5.2
Beef round + Mediterranean salad	983.3 ± 44.6	1071.6 ± 48.5^{D}	9.0	21.0
Beef round + Red cabbage salad	1626.2 ± 73.5	1074.8 ± 48.6^{D}	− 33.9	20.8
Chicken	91.7 ± 4.2	669.8 ± 30.3^{B}	630.4	–
Chicken + Grape juice	2533.5 ± 114.6	457.7 ± 20.7^{C,D}	− 81.9	31.7
Chicken + Orange juice	3853.1 ± 174.4	1206.4 ± 54.6^{A}	− 68.7	− 80.1
Chicken + Red wine	295.1 ± 13.4	214.5 ± 9.7^{G,H}	− 27.3	68.0
Chicken + Hardaliye	4487.1 ± 203.0	306.2 ± 13.8^{F}	− 93.2	54.3
Chicken + Shalgam	169.5 ± 7.8	153.1 ± 6.9^{H}	− 9.7	77.1
Chicken + Pomegranate juice	1566.7 ± 71.0	183.4 ± 8.3^{G,H}	− 88.3	72.6
Chicken + Ayran	147.5 ± 6.8	232.0 ± 10.5^{G}	57.3	65.4
Chicken + Coban salad	728.6 ± 33.0	373.2 ± 16.9^{E,F}	− 48.8	44.3
Chicken + Mediterranean salad	806.3 ± 36.6	412.2 ± 18.7^{D,E}	− 48.9	38.5
Chicken + Red cabbage salad	1449.2 ± 65.5	338.1 ± 15.3^{F}	− 76.7	49.5

Table 1. MDA levels (µg) before and after in vitro digestion. Statistical comparisons were conducted only for post-digestion values to evaluate the effects of co-digestion with beverages and salads. Different capital letters indicated significant differences between samples ($p < 0.05$). The increase/decrease percentages after digestion compared to pre-digestion were calculated and negative values indicate decrease and positive values indicate increase.

digestion. Post-digestion MDA levels in samples co-digested with chicken breast compared to chicken breast alone were significantly reduced by all beverages and salads except orange juice ($p < 0.05$). Shalgam (77.1%), pomegranate juice (72.6%), and red wine (68.0%) were the most effective, while orange juice increased MDA by 80.1%.

The GO levels of beef round and chicken breast samples before and after digestion are shown in Table 2. The GO level in beef round meat increased significantly after digestion, rising from 11.2 ± 0.5 µg to 87.7 ± 4.0 µg, representing a 683% increase. Except for red cabbage salad and hardaliye, all beverages and salads resulted in higher post-digestion GO levels compared to pre-digestion. Grape juice co-digestion caused the highest increase in GO up by 1095.6% post-digestion, compared to beef round alone. Post-digestion GO levels were higher with grape juice, hardaliye, orange juice, pomegranate juice, and red wine ($p < 0.05$), while ayran and shalgam had no significant effect. All salads significantly reduced GO levels after digestion, with Mediterranean, coban, and red cabbage salads inhibiting GO by 62.7%, 57.2%, and 53.6%, respectively. Similarly, in chicken breast, GO levels increased from 7.2 ± 0.3 µg to 98.9 ± 4.5 µg post-digestion, a 1273.6% increase. Post-digestion GO levels were increased by co-digestion with all beverages and salads, with the exception of hardaliye and red cabbage salad. Grape juice resulted in the highest increase (856.2%), while red wine, orange juice, and pomegranate juice caused the lowest increases. Ayran significantly lowered post-digestion GO levels (42.0% inhibition). All salads reduced GO levels, with coban salad (69.4%), red cabbage (67.7%), and Mediterranean salad (62.1%) being the most effective.

The MGO levels of beef round and chicken breast samples before and after digestion are shown in Table 3. MGO levels in beef rounds increased significantly after digestion, from 21.5 ± 1.0 µg to 35.1 ± 1.6 µg (63.3%). Except for hardaliye, all beverages and salads resulted in increased MGO levels post-digestion, with the highest increase observed in orange juice (437.4%). Post-digestion MGO levels were higher than in beef round alone (35.1 ± 1.6 µg) when co-digested with all beverages and salads except shalgam, hardaliye, and Mediterranean salad ($p < 0.05$). Among the beverages, orange juice caused the greatest increase (595.2%), resulting in MGO levels of 244.0 ± 11.0 µg, while red cabbage salad led to the smallest increase (43.0%), resulting in 50.2 ± 2.3 µg. In chicken breast, MGO levels rose from 4.8 ± 0.2 µg to 55.8 ± 2.5 µg post-digestion, reflecting a 1062.5% increase. All salads and beverages increased MGO levels compared to pre-digestion. MGO levels were higher when co-digested with grape juice, orange juice, ayran, red wine, pomegranate juice, hardaliye, and coban salad compared to chicken breast alone (55.8 ± 2.5 µg) ($p < 0.05$). Mediterranean and red cabbage salads did not significantly affect post-digestion MGO levels, while shalgam significantly lowered them by almost half.

Samples	GO (μg)/80 g meat + 150 g salad or 200 mL beverage		Increase/decrease and inhibition % after digestion	
	Before digestion (mean ± SD)	After digestion (mean ± SD)	Increase/decrease	Inhibition
Beef round	11.2 ± 0.5	87.7 ± 4.0^{D}	683.0	–
Beef round + Grape juice	768.6 ± 34.7	1048.5 ± 47.4^{A}	36.4	− 1095.6
Beef round + Orange juice	87.0 ± 3.9	189.0 ± 8.6^{C}	117.2	− 115.5
Beef round + Red wine	55.0 ± 2.5	145.9 ± 6.6^{C}	165.3	− 66.4
Beef round + Hardaliye	539.4 ± 24.3	348.4 ± 15.8^{B}	− 35.4	− 297.3
Beef round + Shalgam	25.2 ± 1.1	51.0 ± 2.3^{D,E}	102.4	41.8
Beef round + Pomegranate juice	91.0 ± 4.1	177.8 ± 8.0^{C}	95.4	− 102.7
Beef round + Ayran	21.2 ± 0.9	60.6 ± 2.7^{D,E}	185.8	30.9
Beef round + Coban salad	27.7 ± 1.2	37.5 ± 1.7^{E}	35.0	57.2
Beef round + Mediterranean salad	30.7 ± 1.4	32.7 ± 1.5^{E}	6.5	62.7
Beef round + Red cabbage salad	44.1 ± 2.0	40.7 ± 1.8^{E}	− 7.9	53.6
Chicken	7.2 ± 0.3	98.9 ± 4.5^{E}	1273.6	–
Chicken + Grape juice	764.6 ± 34.5	945.7 ± 42.8^{A}	23.7	− 856.2
Chicken + Orange juice	83.0 ± 3.7	189.8 ± 8.6^{D}	128.7	− 91.9
Chicken + Red wine	51.0 ± 2.3	188.2 ± 8.5^{D}	269.0	− 90.3
Chicken + Hardaliye	535.4 ± 24.1	315.8 ± 14.3^{B}	− 41.0	− 219.3
Chicken + Shalgam	21.2 ± 0.9	60.6 ± 2.7^{E,F,G}	185.8	38.7
Chicken + Pomegranate juice	87.0 ± 3.9	262.3 ± 11.9^{C}	201.5	− 165.2
Chicken + Ayran	17.2 ± 0.7	57.4 ± 2.6^{F,G}	233.7	42.0
Chicken + Coban salad	23.7 ± 1.1	30.3 ± 1.4^{G}	27.8	69.4
Chicken + Mediterranean salad	26.7 ± 1.2	37.5 ± 1.7^{G}	40.1	62.1
Chicken + Red cabbage salad	40.1 ± 1.8	31.9 ± 1.5^{G}	− 20.4	67.7

Table 2. GO levels (μg) before and after in vitro digestion. Statistical comparisons were conducted only for post-digestion values to evaluate the effects of co-digestion with beverages and salads. Different capital letters indicated significant differences between samples ($p < 0.05$). The increase/decrease percentages after digestion compared to pre-digestion were calculated and negative values indicate decrease and positive values indicate increase.

Discussion

This study demonstrated significant increases in malondialdehyde (MDA), glyoxal (GO), and methylglyoxal (MGO) levels during the in vitro digestion of beef and chicken. Notably, beverages such as pomegranate juice, shalgam, and red wine exhibited strong antioxidative effects by reducing MDA levels, while others like grape juice and orange juice exacerbated lipid oxidation. Similarly, red cabbage and Mediterranean salads significantly reduced GO and MGO levels, whereas coban salad was less effective. These findings underscore the varying impacts of dietary components on oxidative stress markers during digestion, highlighting the potential for targeted dietary strategies to mitigate oxidative damage.

The gastrointestinal system plays a central role in facilitating lipid oxidation, acting as a bioreactor where iron, oxygen, and emulsified lipids contribute to the formation of harmful oxidative products[26,27]. This oxidation, along with sugar degradation, produces GO and MGO, precursors of advanced glycation end products (AGEs)[3], which are accelerated by digestion[26]. Consistent with previous studies[4,6], this study observed significant increases in MDA, GO, and MGO levels in both beef and chicken during digestion. The higher increase in chicken may be due to its lower thiamine content, suggesting that thiamine and similar compounds may inhibit oxidation[29].

MDA serves as an indicator of lipid peroxidation, while GO and MGO reflect AGE formation driven by lipid and sugar degradation[3,7]. Meat's fatty acids and iron content make it susceptible to oxidative damage during digestion[7], but antioxidant-rich beverages and salads, due to phenolic compounds, can mitigate this by inhibiting lipid oxidation and AGE formation[29,30]. Previous in vivo human[9,10], and animal studies[26,31], and in vitro studies[17–19,32–34] show that lipid oxidation can be reduced by antioxidant-rich foods.

Grapes, rich in phenolic compounds such as anthocyanins, resveratrol, and quercetin, are widely recognized for their antioxidant potential. These compounds are often used in meat processing, especially from wine by-products[8]. While there is limited research on the effect of grape juice on lipid oxidation during digestion, human studies confirm that it reduces oxidative stress markers such as MDA[9]. Red wine, which is richer in bioactive compounds and antioxidants than grape juice[11], has been shown to inhibit lipid oxidation in meat[12,32]. Pomegranate, which is rich in anthocyanins and flavanols, has three times the antioxidant activity of red wine[8,13]. Traditional fermented beverages such as hardaliye (from red grapes) and shalgam (from black carrots) have also shown strong antioxidant potential due to their phenolic and anthocyanin content[14,15]. In this study, red wine, pomegranate juice, hardaliye, and shalgam significantly reduced MDA levels in beef and chicken, while grape juice increased them. The increased lipid oxidation in beef may be due to higher iron levels, as iron reactivity is increased when chelated with grape juice[31]. In addition, red wine, pomegranate juice, and hardaliye increased

Samples	MGO (µg)/80 g meat + 150 g salad or 200 mL beverage		Increase/decrease and inhibition % after digestion	
	Before digestion (mean ± SD)	After digestion (mean ± SD)	Increase/decrease	Inhibition
Beef round	21.5 ± 1.0	35.1 ± 1.6^{G}	63.3	–
Beef round + Grape juice	149.1 ± 6.8	152.3 ± 6.9^{B}	2.1	− 333.9
Beef round + Orange juice	45.4 ± 2.1	244.0 ± 11.0^{A}	437.4	− 595.2
Beef round + Red wine	111.2 ± 5.1	120.4 ± 5.5^{C}	8.3	− 243.0
Beef round + Hardaliye	79.3 ± 3.6	32.7 ± 1.5^{G}	− 58.8	6.8
Beef round + Shalgam	27.5 ± 1.3	36.7 ± 1.7^{E,G}	33.1	− 4.6
Beef round + Pomegranate juice	71.3 ± 3.3	87.7 ± 4.0^{D}	23.0	− 149.9
Beef round + Ayran	57.4 ± 2.6	98.9 ± 4.5^{D}	72.3	− 181.8
Beef round + Coban salad	43.9 ± 2.0	62.2 ± 2.8^{E}	41.7	− 77.2
Beef round + Mediterranean salad	26.0 ± 1.2	31.1 ± 1.4^{G}	19.6	11.4
Beef round + Red cabbage salad	23.0 ± 1.1	50.2 ± 2.3^{E,F}	118.3	− 43.0
Chicken	4.8 ± 0.2	55.8 ± 2.5^{F}	1062.5	–
Chicken + Grape juice	132.4 ± 6.0	151.5 ± 6.9^{A}	14.4	− 171.5
Chicken + Orange juice	28.7 ± 1.3	130.8 ± 5.9^{B}	355.7	− 134.4
Chicken + Red wine	94.5 ± 4.3	110.0 ± 5.0^{C}	16.4	− 97.1
Chicken + Hardaliye	62.6 ± 2.8	87.7 ± 4.0^{D}	40.1	− 57.2
Chicken + Shalgam	10.8 ± 0.5	25.5 ± 1.2^{G}	136.1	54.3
Chicken + Pomegranate juice	54.6 ± 2.5	106.8 ± 4.8^{C}	95.8	− 91.4
Chicken + Ayran	40.7 ± 1.8	113.2 ± 5.1^{C}	178.1	− 102.9
Chicken + Coban salad	27.2 ± 1.2	71.8 ± 3.3^{E}	164.0	− 28.7
Chicken + Mediterranean salad	9.3 ± 0.4	56.6 ± 2.6^{F}	508.6	− 1.4
Chicken + Red cabbage salad	6.3 ± 0.3	47.0 ± 2.1^{F}	646.0	15.8

Table 3. MGO levels (µg) before and after in vitro digestion. Statistical comparisons were conducted only for post-digestion values to evaluate the effects of co-digestion with beverages and salads. Different capital letters indicated significant differences between samples ($p < 0.05$). The increase/decrease percentages after digestion compared to pre-digestion were calculated and negative values indicate decrease and positive values indicate increase.

GO and MGO levels after digestion, whereas shalgam significantly reduced MGO. Beverages containing glucose and fructose can increase GO and MGO levels, with fructose leading to greater MGO production[35]. Orange juice also increased GO and MGO levels due to its fructose and ascorbic acid content[36]. Shalgam's lower sugar content during fermentation likely contributed to its lower GO and MGO levels compared to other beverages.

Ayran, a traditional Turkish beverage made from yoghurt, has not been extensively studied for its effects on lipid oxidation in meat during in vitro digestion. However, dairy products are known for their antioxidant properties. For example, Lamothe et al.[33] found that dairy products reduced lipid oxidation by approximately 60%, and casein proteins in particular can inhibit this process[34]. In this study, MDA levels decreased in both beef and chicken, probably due to the antioxidant activity of the dairy proteins in ayran. Ayran had minimal effect on GO levels in beef but increased MGO levels. In chicken, while GO levels were low, MGO levels were higher. Its low sugar content in comparison to other beverages and its partial suppression of lipid oxidation could account for the comparatively lower production of GO and MGO in ayran. These results suggest that ayran has some antioxidant capacity, but its influence on GO and MGO production is complex and depends on the type of meat.

Vegetables, like fruits, are rich in antioxidants and can prevent oxidation in meat products[16]. Red cabbage extract has been shown to reduce lipid oxidation in fish, and its anthocyanins are more stable than those from grapes[17]. In an in vitro study on postprandial oxidative stress, spinach, broccoli, cabbage, and red peppers were the most effective in preventing lipid oxidation in turkey meat, while tomatoes and green peppers showed the least activity, with green peppers even showing prooxidant effects in the stomach[18]. A Mediterranean salad combined with grilled turkey breast inhibited lipid oxidation[19], and a Greek salad reduced MDA formation by 90% during digestion[18]. In this study, red cabbage and Mediterranean salads reduced MDA levels in all meats, while coban salad was less effective, possibly due to the pro-oxidant effect of green peppers and the lack of vinegar, which has antioxidant properties[37]. Vegetables in salads also have lower AGE levels compared to other foods, and the inclusion of lemon and olive oil likely contributed to the reduced formation of GO and MGO[38,39].

This study has several limitations that should be considered. First, the use of an in vitro digestion model, while comprehensive, may not fully replicate the complex interactions that occur in the human gastrointestinal tract. This limits the direct applicability of the findings to real-life dietary scenarios. Additionally, the long-term effects of these beverages and salads on oxidative stress markers in vivo remain unexplored. Future research should focus on in vivo studies to validate these results and examine dose-dependent effects of dietary components on oxidative markers.

Despite these limitations, this study provides valuable insights into the relationship between diet and meat quality. The findings suggest that antioxidant-rich dietary components such as shalgam, pomegranate juice, and Mediterranean salads could be integrated into meal preparations to mitigate oxidative stress and improve food quality. These results have implications not only for consumer dietary practices but also for the food industry in developing functional food products to enhance nutritional value and health outcomes.

Conclusion

This study provides compelling evidence that incorporating antioxidant-rich beverages and salads can significantly reduce lipid oxidation and the formation of MDA, GO, and MGO in meats during in vitro gastrointestinal digestion. The protective effects observed with red wine, pomegranate juice, and various salads underscore their potential as functional food components that enhance meat quality. However, the study also reveals that the effectiveness of these antioxidants varies based on the type of meat and the specific ingredients used, suggesting that dietary strategies for reducing oxidative stress should be tailored accordingly. Future research should further explore the mechanisms behind these protective effects and their implications for human health.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 21 February 2025; Accepted: 16 April 2025
Published online: 16 May 2025

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Author contributions

G.S.: conceptualization, data curation, formal analysis, investigation, methodology, resources, visualization, writing-original draft, writing-review and editing. G.A.: conceptualization, methodology, project administration, supervision, writing-original draft, writing-review and editing. M.Y.: conceptualization, data curation, formal analysis, investigation, methodology, resources, visualization, writing-original draft, writing-review and editing.

Funding

No funds, grants, or other support was received.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to G.S. or M.Y.