

Investigation of *in vitro* formation of advanced lipoxidation end products and advanced glycation end products precursors in high-fat processed meat products

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Abstract

The precursors glyoxal (GO) and methylglyoxal (MGO) of advanced glycation end products (AGEs) or advanced lipoxidation end products (ALEs) occur exogenously and endogenously. These α -dicarbonyl compounds, GO and MGO, can be formed from Maillard reactions during food processing as well as protein and lipid peroxidation. The purpose of the present study was to determine and evaluate the *in vitro* formation of AGEs and ALEs precursors, GO and MGO, in high-fat processed meat products. Before digestion, GO and MGO amounts in the samples ranged between 59.0 and 81.0 $\mu\text{g}/100\text{ g}$ and between 11.7 and 47.0 $\mu\text{g}/100\text{ g}$, respectively. After *in vitro* digestion, GO and MGO formation ranged between 147.0 and 514.1% and 156.0 and 6912.3%, respectively. It is believed that prooxidant components, such as oxygen, enzymes, or heme-proteins, in the gastrointestinal tract promote lipid oxidation; therefore, GO and MGO can be formed. A higher rate of MGO increase was observed in starch containing meatball (chicken) and nugget samples in the gastrointestinal tract. It is thought that the carbohydrates in the meat samples may have contributed to the amount of GO and MGO formed. Thus, reducing fat and starch may produce low levels of GO and MGO formation in the foods during processing or digestion.

Keywords: meat; fat; glyoxal; methylglyoxal; *in vitro*.

Practical Application: Reducing fat, starch, and salt may produce low levels of GO and MGO formation in the foods during processing or digestion.

1 Introduction

The precursors glyoxal (GO) and methylglyoxal (MGO) of advanced glycation end products (AGEs) or advanced lipoxidation end products (ALEs) occur exogenously and endogenously (Uribarri et al., 2010; Sharma et al., 2015; Çatak et al., 2022). These α -dicarbonyl compounds (α -DCs), GO and MGO, can be formed from Maillard reactions (MRs) during food processing as well as protein oxidation and lipid peroxidation. MRs begin with nonenzymatic reactions between reducing sugars and an amino group of proteins, amino acids, nucleic acids, peptides, and lipids, leading to the generation of unstable Schiff bases. The products then undergo unstable intermolecular Amadori rearrangements and become the more-stable Amadori/Heyns products. They then degrade into highly reactive α -DCs, such as GO, MGO, and 3-deoxyglucosone (3-DG) (Poulsen et al., 2013). The final AGEs, such as N- ϵ -carboxymethyllysine, N- ϵ -carboxyethyllysine, and pentosidine, can be formed from lysine, arginine, histidine, and cysteine residues and the amino groups of proteins (Henle, 2005; Luevano-Contreras & Chapman-Novakofski, 2010). GO and MGO are the most abundant α -DCs found in processed foods and biological systems (Liu et al., 2011). In the human body, GO and MGO can occur during lipid peroxidation, glucose autoxidation, and the polyol pathway (Luevano-Contreras & Chapman-Novakofski, 2010). These α -DC compounds are up to 20,000 times more reactive than glucose in glycation reactions (Thornalley, 2005). MR products are partially absorbed by the intestinal system. High amounts

of α -DC compounds are found in the circulation system of type 2 diabetes mellitus (T2DM) patients. GO and MGO covalently bind to the amino groups of the insulin protein in pancreatic cells, leading to AGEs. Hence, insulin resistance develops, and cellular glucose uptake decreases (Nowotny et al., 2015). Some studies have shown that high levels of MGO-derived AGEs are detected in circulation systems in T2DM, Parkinson's disease, Alzheimer's disease, aging, renal failure, and arthritis (Rabbani & Thornalley, 2014).

Generally, α -DCs can be formed in lipid-rich and carbohydrate-rich foods, beverages, fume smoked meat products, and some other baked foods (Uribarri et al., 2010; Papetti et al., 2014). A significant correlation was reported between fat content and MGO level and the occurrence of dietary AGEs (Wang, 2019; Sun et al., 2015). Fujioka and Shibamoto have reported that the GO and MGO content is <1.2 and $<0.8\text{ mg/kg}$ in raw fat samples, and 0.8-4.0 and 0.2-1.3 mg/kg in cooked fat samples, respectively. As reported in their study, cooking increases the amounts of both GO and MGO (Fujioka & Shibamoto, 2004).

Meat is an important food in human nutrition because it contains high-quality protein, lipids, and iron. Because of its nutritional content, meat can easily deteriorate from chemical and enzymatic degradation during production and storage. GO and MGO are the aldehyde groups of lipid peroxidation products (Vistoli et al., 2013). It has been reported that the amount of

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GO and MGO from meat products is generally lower than that in other food groups because meat and meat products contain lower rates of unsaturated fats (Maasen et al, 2021).

There are limited studies on the formation of the precursors of AGE and ALE products *in vitro*. Sansano et al. (2017) have found increased amounts of acrylamide in French fries and chips after digestion. Papetti et al. (2013) have reported a decreased amount of GO and MGO in vinegar; however, the results of that same study showed an increased amount of GO and MGO in soy sauce *in vitro* (Papetti et al., 2014). There are limited studies on the increase in lipid peroxidation products during digestion of fish and pork. Although meat products are important in our diets, it is also important to recognize the harmful compounds, such as GO and MGO, contained in meat that lead to the formation of AGEs or ALEs; however, determining the amounts of GO and MGO in meat and meat products alone is not sufficient because the amount formed *in vitro* is important in terms of determining the daily intake. The final amount of GO and MGO will affect final amounts of AGEs and ALEs (Nieva-Echevarría et al., 2020). The purpose of the present study was to determine and evaluate the *in vitro* formation of GO and MGO in high-fat processed meat products using a simulated gastrointestinal digestive system.

2 Materials and methods

2.1 Materials

GO (%40), MGO (%40), 4-nitro-1,2-phenylenediamine, methanol (MeOH), acetonitrile (ACN), sodium acetate, alpha-amylase (1.5 U/mg), pepsin (≥ 250 units/mg solid), pancreatin (8 $\times$ USP specifications), lipase (100-500 units/mg protein), NaHCO₃, CaCl₂·2H₂O, KCl, NaCl, serum albumin, bile salts, urea, uric acid, and mucin were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Sampling

The processed meat products used in the present study were obtained from different supermarkets in Afyonkarahisar, Turkey. The processed type of the high-fat meat products are given in Table 1.

2.3 Fat, salt and starch analysis

The fat content in the samples was determined using the Soxhlet extraction method described by Uylaşer & Başoğlu (2000). The salt content in the samples was determined using the method described by Kuyumcu & Yurttagül (2000). The starch amount in processed meat products was determined as described by Goñi et al. (1996). Briefly: The sample (0.1 g) was weighed into a 50-mL plastic falcon tube and 2 mL KOH (2 M) solution and 8 mL sodium acetate (1 M, pH 3.8) were added. Thermostable α -amylase and amyloglucosidase (0.1 mL) enzymes were used for starch hydrolysis (50 °C for 30 min), after which 0.1 mL sample was treated with 3.0 mL glucose oxidase/ peroxidase solution and incubated in a water bath at 50 °C for 30 min. The absorbance was measured at 510 nm using a UV-1280 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

2.4 Extraction and derivatization of GO and MGO

The GO and MGO in the samples were extracted using the method determined by Cengiz et al. (2020). Five g sample was weighed into a 50-mL plastic falcon tube and 25 mL MeOH was added. The sample was then vortexed for 5 min and centrifuged at 8000 rpm for 5 min, after which 1 mL centrifuged liquid sample was added to a 10 mL glass tube with 1 mL sodium acetate buffer (0.1 M, pH 3) and 0.5 mL derivatization solution (4-nitro-1,2-phenylenediamine in 1% MeOH) were mixed and incubated at 70 °C for 20 min. The final samples were then filtered through a 0.45 μ m cellulose acetate filter and injected into the high-performance liquid chromatography (HPLC) apparatus.

Table 1. Sample types with their processing type, amount of fat, and starch.

Sample No	Product Name	Salt (g/100 g)	Fat Content (g/100 g)	Starch Content (g/100 g)
1	Döner, meat, roasted, unpacked	1.2 $\pm$ 0.2	25.8 $\pm$ 0.6	0
2	Döner, meat, roasted, unpacked	1.7 $\pm$ 0.3	23.8 $\pm$ 0.6	0
3	Döner, meat, roasted, unpacked	1.3 $\pm$ 0.1	25.6 $\pm$ 1.0	0
4	meatball, chicken, precooked	1.4 $\pm$ 0.2	17.7 $\pm$ 0.8	4.9 $\pm$ 0.3
5	meatball, chicken, precooked	1.3 $\pm$ 0.1	18.2 $\pm$ 0.6	5.0 $\pm$ 0.1
6	meatball, chicken, precooked	1.7 $\pm$ 0.2	13.7 $\pm$ 0.8	9.2 $\pm$ 0.7
7	Nuggets, chicken, precooked	1.5 $\pm$ 0.2	20.4 $\pm$ 0.8	24.1 $\pm$ 0.5
8	Nuggets, chicken, precooked	1.9 $\pm$ 0.1	15.3 $\pm$ 1.3	16.0 $\pm$ 1.8
9	Nuggets, chicken, precooked	1.8 $\pm$ 0.2	15.2 $\pm$ 0.6	23.4 $\pm$ 0.9
10	Kavurma, meat,roasted	2.0 $\pm$ 0.3	30.5 $\pm$ 0.6	0
11	Kavurma, meat,roasted	1.8 $\pm$ 0.3	29.5 $\pm$ 0.6	0
12	Kavurma, meat,roasted	2.8 $\pm$ 0.3	29.4 $\pm$ 0.8	0
13	Pastirma, air-dried, cured, pressed beef cut	4.8 $\pm$ 0.4	8.7 $\pm$ 0.6	0
14	Pastirma, air-dried, cured, pressed beef cut	4.6 $\pm$ 0.2	6.0 $\pm$ 0.9	0
15	Pastirma, air-dried, cured, pressed beef cut	4.4 $\pm$ 0.2	7.3 $\pm$ 1.0	0

Notes: Values are means $\pm$ range, n = 3.

2.5 HPLC determination of GO and MGO

The HPLC conditions for detecting GO and MGO as described by Cengiz et al. (2020) were used. The HPLC system comprised a Shimadzu LC 20AT pump with a SPD-20A UV/VIS detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase consisted of MeOH:water:ACN (42:56:2 v/v/v). The wavelength was set 255 nm and an Inersil ODS-3 column (GL Sciences, Japan) was used with a flow rate of 1 mL/min. The column oven temperature was 30 °C.

2.6 *In vitro* formation of GO and MGO

The formation of GO and MGO was measured using a simulated *in vitro* human digestive system as previously described by Yaman & Mızrak (2019). The solutions in the human digestive system, such as saliva, gastric, duodenal, and bile juices were prepared as shown in Figure 1.

Five grams of each sample were mixed with 5 mL saliva in a 50-mL falcon tube and incubated for 5 min at 37 °C while shaking in a water bath. Next, 10 mL gastric juice was added and incubated for 30 min at 37 °C while shaking in a water bath, after which 5 mL bile juice was adjusted to pH 7, and 10 mL duodenal juice was added. This intestinal phase was incubated for 2 h at 37 °C while shaking in a water bath. The final volume was then adjusted with deionized water to 50 mL and the solution was centrifuged for 5 min at 8000 rpm.

2.7 Statistical analyses

The mean value ($n = 3$) was provided with the standard deviation. Significant differences were calculated using the analysis of variance (ANOVA) Tukey's test ($p < 0.05$).

3 Results and discussion

The HPLC chromatograms of GO and MGO standards and sample 7 (nuggets, chicken, precooked) are shown in Figure 2 and Figure 3, respectively. The fat and starch contents in processed meat products are shown in Table 1, and the amount of GO and MGO before and after digestion and the rate of formation are shown in Table 2.

The fat content in processed meat ranged between 6.0 and 29.5 g/100 g. As can be seen from the table, among the processed meat samples, high amounts of fat were determined in Kavurma samples (29.4-30.5 g/100 g), while low amounts were determined in the pastirma samples (6.0-8.7 g/100 g). The initial measured amount of GO and MGO in the processed meat samples ranged from 59.0 to 81.0 µg/100 g and from 11.7 to 47.0 µg/100 g, respectively.

Both microbiological degradation and oxidation can occur in meat and meat products. The products of the lipid peroxidation reaction can easily react with proteins, causing both sensory changes and loss of nutritional value (Guyon et al., 2016). Meat lipids consist of triglycerides and phospholipids containing saturated fatty acids, monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). The unsaturated fatty acid in meat contains one or two double-chain fatty acids. Oleic (C18:1), linoleic (LA, C18:2 $n - 6$), and linolenic (C18:3 $n - 3$) are the most common unsaturated fatty acids in beef. Lipid peroxidation usually occurs as a result of degradation of PUFAs during food processing and cooking and forms carbonyls and hydrocarbon compounds (Pereira & Abreu, 2018). Lipid peroxidation occurs between PUFAs and oxygen. Autoxidation, enzymatic-catalyzed oxidation, and photo-oxidation are the main methods by which lipids oxidize. Prooxidant components, such

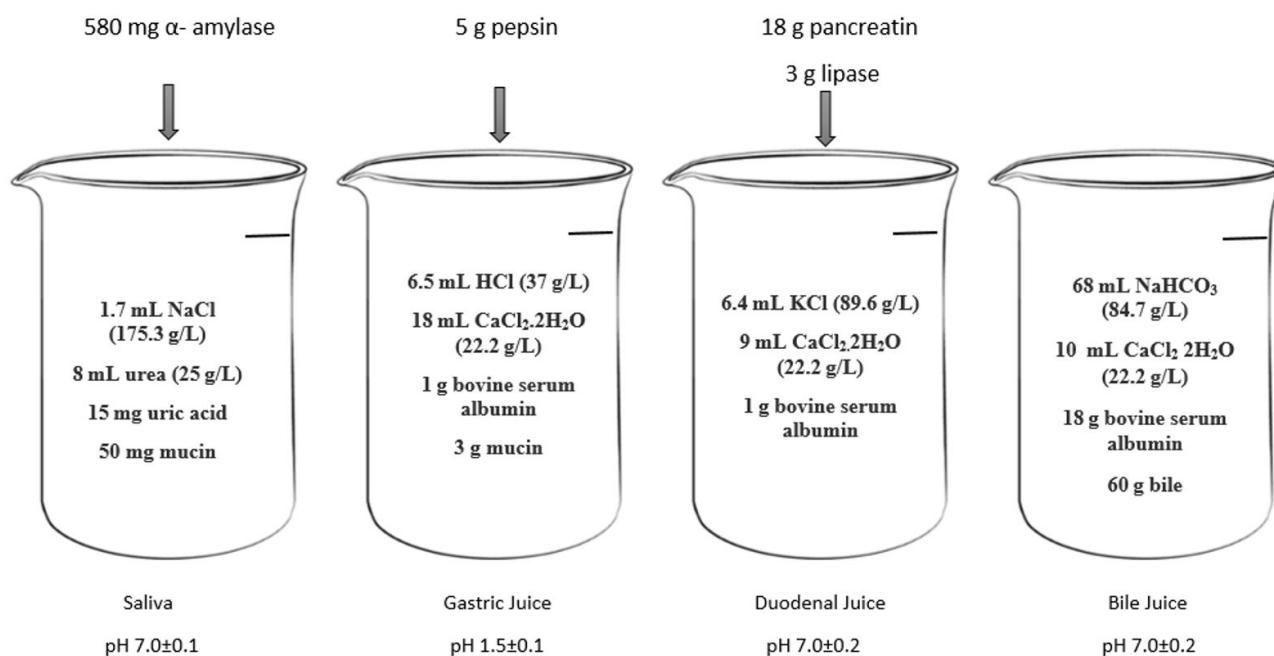


Figure 1. Number of enzymes and concentrations of saliva, gastric, duodenal, and bile juices used in the *in vitro* human digestion model.

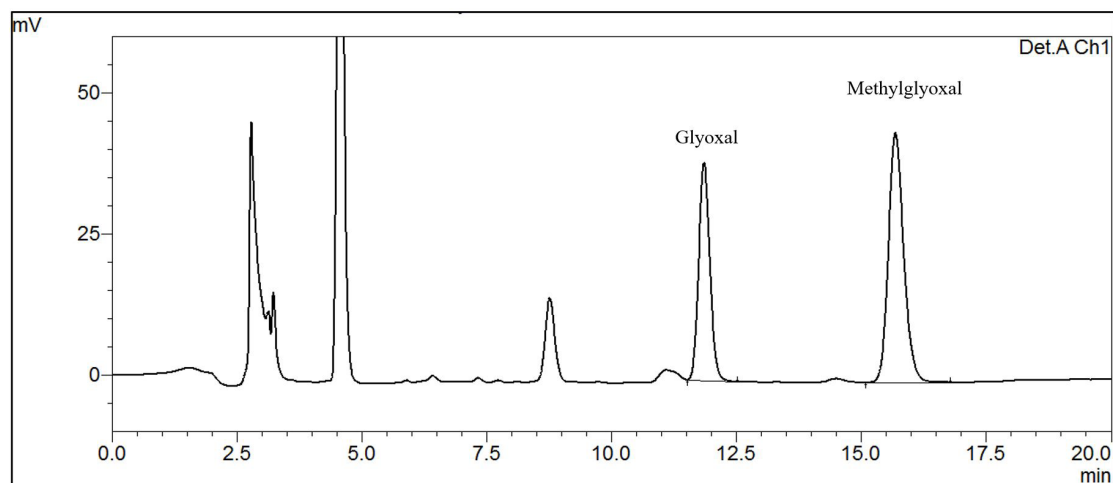


Figure 2. High-performance liquid chromatography standard chromatogram of glyoxal and methylglyoxal.

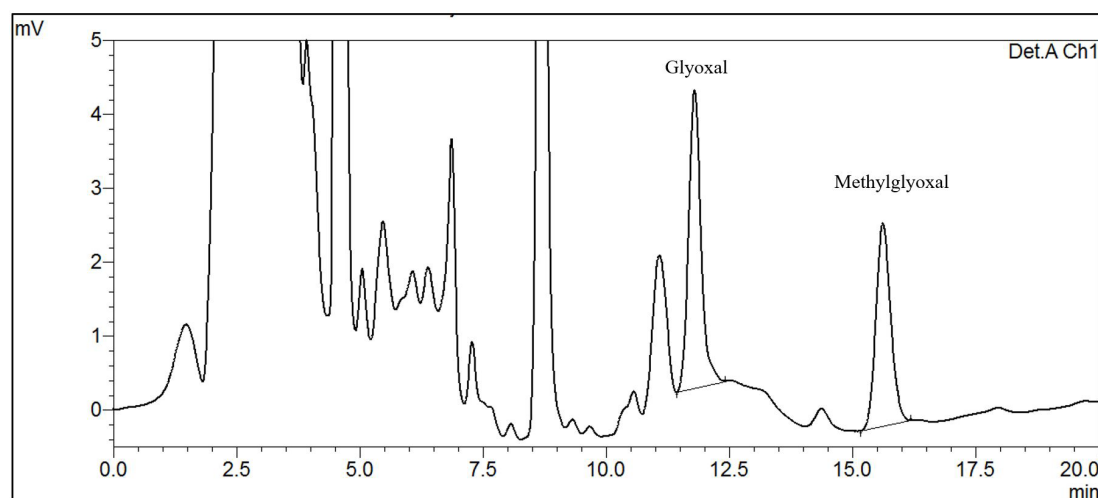


Figure 3. High-performance liquid chromatography chromatogram of glyoxal and methylglyoxal in sample 7.

Table 2. GO and MGO formation in samples before and after *in vitro* digestion.

Sample No.	Product	Initial Value	After Digestion	Initial Value	After Digestion	Formation %	
		GO ($\mu\text{g}/100\text{ g}$)	GO ($\mu\text{g}/100\text{ g}$)	MGO ($\mu\text{g}/100\text{ g}$)	MGO ($\mu\text{g}/100\text{ g}$)	GO	MGO
1	Döner, meat, roasted, unpacked	81.0 $\pm$ 3.6 ^a	232.0 $\pm$ 6.0 ^b	11.7 $\pm$ 1.2 ^a	84.7 $\pm$ 4.0 ^b	286.4	725.7
2	Döner, meat, roasted, unpacked	74.7 $\pm$ 2.5 ^a	282.7 $\pm$ 6.4 ^b	26.0 $\pm$ 1.0 ^a	113.7 $\pm$ 6.7 ^b	378.6	437.2
3	Döner, meat, roasted, unpacked	78.0 $\pm$ 3.6 ^a	162.3 $\pm$ 4.0 ^b	24.7 $\pm$ 1.5	256.7 $\pm$ 7.2 ^b	208.1	1040.5
4	meatball, chicken, precooked	74.0 $\pm$ 2.6 ^a	290.7 $\pm$ 5.9 ^b	19.0 $\pm$ 1.0 ^a	1313.3 $\pm$ 37.3 ^b	392.8	6912.3
5	meatball, chicken, precooked	63.7 $\pm$ 3.2 ^a	313.7 $\pm$ 5.7 ^b	47.0 $\pm$ 2.6 ^a	337.7 $\pm$ 9.6 ^b	492.7	718.4
6	meatball, chicken, precooked	59.0 $\pm$ 2.6 ^a	303.3 $\pm$ 6.1 ^b	31.0 $\pm$ 2.0 ^a	1853.7 $\pm$ 49.7 ^b	514.1	5979.6
7	Nuggets, chicken, precooked	69.3 $\pm$ 4.0 ^a	210.3 $\pm$ 5.0 ^b	36.3 $\pm$ 2.3 ^a	134.3 $\pm$ 7.1 ^b	303.4	369.7
8	Nuggets, chicken, precooked	74.0 $\pm$ 3.6 ^a	154.3 $\pm$ 6.7 ^b	23.7 $\pm$ 1.5 ^a	277.3 $\pm$ 10.1 ^b	208.6	1171.8
9	Nuggets, chicken, precooked	80.7 $\pm$ 3.1 ^a	191.0 $\pm$ 6.1 ^b	31.7 $\pm$ 5.7 ^a	149.0 $\pm$ 4.0 ^b	236.8	470.5
10	Kavurma, meat, roasted	71.3 $\pm$ 4.2 ^a	292.3 $\pm$ 5.9 ^b	27.7 $\pm$ 2.1 ^a	71.0 $\pm$ 4.0 ^b	409.8	256.6
11	Kavurma, meat, roasted	66.0 $\pm$ 3.6 ^a	291.7 $\pm$ 7.6 ^b	30.3 $\pm$ 1.5 ^a	92.0 $\pm$ 3.6 ^b	441.9	303.3
12	Kavurma, meat, roasted	76.3 $\pm$ 4.2 ^a	181.0 $\pm$ 7.2 ^b	27.0 $\pm$ 1.0 ^a	85.0 $\pm$ 4.6 ^b	237.1	314.8
13	Pastırma, air-dried, cured, pressed beef cut	68.3 $\pm$ 3.5 ^a	223.0 $\pm$ 5.3 ^b	23.0 $\pm$ 2.0 ^a	80.3 $\pm$ 5.0 ^b	326.3	349.3
14	Pastırma, air-dried, cured, pressed beef cut	88.3 $\pm$ 3.6 ^a	261.7 $\pm$ 7.8 ^b	30.7 $\pm$ 2.1 ^a	59.0 $\pm$ 3.6 ^b	296.2	192.4
15	Pastırma, air-dried, cured, pressed beef cut	66.0 $\pm$ 3.6 ^a	97.0 $\pm$ 5.6 ^b	36.3 $\pm$ 2.1 ^a	56.7 $\pm$ 2.3 ^b	147.0	156.0

Notes: GO = glyoxal; MGO = methylglyoxal. Values are means $\pm$ range, n = 3. The different letters in the same rows for GO and MGO show that there are statistical differences (analysis of variance $p < 0.05$, Tukey's test).

as oxygen, enzymes, or heme-proteins in meat and meat samples, promote the oxidation processes (Domínguez et al., 2019). GO and MGO, can be formed during food processing by degradation of starch and other sugars as well as protein and lipid oxidation (Vistoli et al., 2013). Depending on the reaction time and increased cooking temperature, long-term storage conditions can lead to the formation of α -DCs in foods (Sharma et al., 2015; Wang, 2019). As observed, lipid peroxidation may cause α -DC formation in meat and meat products resulting from storage and processing conditions (Wang, 2019; Vistoli et al., 2013). Maasen et al. (2021) have reported that the amounts of GO and MGO in meat and meat products are very low (<10 mg/kg) compared to that in other foods. In one study, Hipkiss has reported that the total amount of α -DCs in meat samples is <1 mg/kg and that this amount may vary depending on cooking type and added sugar (Hipkiss, 2005). In another study, creatine in meat products was observed to act as a scavenger for α -DCs, which might explain the low amounts (Treibmann et al., 2019); however, high amounts of MGO were found in zebra-fish and it is believed that long-chain PUFAs, possibly produce MGO through lipid peroxidation (Fujioka & Shibamoto, 2004; Suh et al., 2017). In our samples, the total GO and MGO was ~1 mg/kg or less. As mentioned, GO and MGO can be formed by degradation of starch and lipid peroxidation. As seen from our results, generally, MGO content in the meatball and nugget samples were higher than that in other samples. It is believed that in addition to fat, the presence of starch in these samples contributed to the formation of GO and MGO. Besides, curing conditions such as temperature and pH increase the free amino acid content in fermented sausages. Accordingly, the formation of alpha dicarbonyl products, which are maillard reaction products, can be expected more. Therefore, the curing method may have caused the formation of GO and MGO in pastirma (Li et al., 2021).

3.1 *In vitro*

As seen from the Table 2, after digestion, the amount of GO and MGO were increased compared to their initial values and ranged between 97.0 and 313.7 g/100 g, and 59.0 and 1853.7 g/100 g, respectively. The formation of GO and MGO ranged between 147.0 and 514.1% and 156.0 and 6912.3%, respectively.

There are a limited number of studies on the increase or decrease in GO and MGO in foods *in vitro*. Papetti et al. (2014) have studied the bioaccessibility of free α -DCs in two balsamic vinegars and found that the GO and MGO concentrations are reduced by approximately 30%, and the authors suggest that digestion could reduce the bioaccessibility of the α -DCs in foods. In that study, the authors have also indicated that the α -DCs are reduced during digestion because they may convert to harmful AGEs in the presence of digestive enzymes. Yang et al. (2011) have reported that the decrease in α -DCs during digestion results from a reaction of α -DCs with proteins and suggested that AGEs are formed as the final product. Papetti et al. have shown that the bioaccessible amounts of GO, MGO, and DA (diacetyl) in brewed coffee is reduced by 74, 29, and 67%, respectively, and have suggested that α -DCs are reduced during digestion to yield AGEs because of their reactions with enzymatic digestion proteins. In the same study, Papetti et al. (2014) have shown that

the concentration of GO and MGO in soy sauce increases by 290 and 1000%, respectively, and that the DA concentration in barley coffee increases by 3500%. The reason for these increases and decreases is believed to be related to the food matrix; however, the type of food matrix affected has not been clarified. Unlike the results of these studies, there is evidence of an increase in lipid peroxidation products, such as malondialdehyde (MDA), 4-oxo-2-nonenal (ONE), and hexanal and α -DCs in the *in vitro* studies (Nieva-Echevarría et al., 2020).

As seen from Table 1, the meat and meat products included in the present study contain high amounts of fat ranging from 6.0 to 30.5 g/100 g. Foods of animal origin contain both saturated and unsaturated fats. As discussed, prooxidant components, such as oxygen, enzymes, or heme-proteins, in meat and meat samples promote the oxidation processes; therefore, as a result of lipid peroxidation in meat and meat products, α -DCs may be formed (Domínguez et al., 2019). It has been reported that lipid peroxidation can occur not only in food production but also in gastrointestinal digestion because under gastrointestinal digestion, pro-oxidant factors, such as low gastric pH and the presence of oxygen and iron ions, copper ions, and lipid hydroperoxides, may promote lipid peroxidation in gastrointestinal digestion (Guillén & Ruiz, 2004; Johnson & Decker, 2015). Kuffa et al. (2009) have reported that during digestion in the stomach, lipid peroxidation products, such as hydroperoxides, form in turkey meat. As a result, many harmful compounds may occur from lipid peroxidation during digestion (Halliwell et al., 2000). Many studies have reported that lipid peroxidation products, such as 4-hydroxy-2-nonenal, 4-hydroxy-2-hexenal, MDA, hexanal and GO increases during *in vitro* digestion (Nieva-Echevarría et al., 2020). Van Hecke et al. have conducted a study on the relationship between the amount of fat consumed and the formation of lipid peroxidation products during gastrointestinal digestion (Van Hecke et al., 2014). In that study, 1, 5, and 20% pork fat were added to pork meat. Addition of 5 or 20% fat compared to 1% produces a high amount of lipid peroxidation products during digestion. Larsson et al. have reported that during digestion, the amount of lipid peroxidation products increases as the unsaturated fat content increases (Larsson et al., 2016). Although the meat and meat products used in the present study are known to contain high amounts of saturated fat, they also contain ~50% MUFAs (Turkomp, 2021). As observed, the formation of lipid peroxidation products, such as MDA and α -DCs, in the meat and meat products used in the present study may occur during *in vitro* digestion.

As seen from the Table 2, the increasing rate of MGO in sample 4 and 5 (meatball, chicken, precooked) and sample 8 (Nuggets, chicken, precooked) were observed to be higher compared to other meat samples. When the nutritional contents were evaluated, these samples also contained starch in addition to fat. The starch in these samples ranged between 4.9 and 24.1 g/100 g. Martinez-Saez et al. (2019) have reported that Maillard reaction products form during *in vitro* digestion of an average meal (protein, starch, and fat) and that those products form during digestion because the free amino acids react with free glucose and fructose. During digestion, proteins and starch are hydrolyzed by enzymes. Free amino acids released from the proteins and free sugars are also released from the starch;

therefore, reactive free amino acids react with reactive carbonyl groups leading to Maillard reaction products. It was also reported that the presence of reducing sugar increases DCs resulting from auto-oxidation (Sakai et al., 2002). It is suggested that the starch contained in these examples in addition to the fat contribute to increasing the formation of MGO in the gastrointestinal tract.

Oxidation of lipids can occur by adding salt in the presence of oxygen, both enzymatically and non-enzymatically. Sodium chloride is used as a food additive to preserve and reduce antimicrobial activity in the foods. The addition of salt also reduces antioxidant enzyme activity. The antioxidant enzymes inhibit lipid oxidation (Mariutti & Bragagnolo, 2017). As observed, the addition of salt increases lipid oxidation and inhibition of antioxidant enzymes and thus increases lipid peroxidation. The measurement amount of salt in döner, meatball, nuggets, kavurma and pastirma used in the present study between 1.2-1.7, 1.3-1.7, 1.5-1.9, 1.8-2.8, and 4.4-6.8 g/100 g, respectively. It is seen that the amount of salt in the samples is variable. It is believed that the salt content may contributed to the increased lipid peroxidation.

Generally, in the present study, GO and MGO formation *in vitro* increased by >100%. As observed, carbohydrate and fat together may cause the Maillard reaction in the *in vitro* gastrointestinal system and produce harmful AGE precursors. As AGE precursors, MGO and GO react with DNA, lipids, and reactive free amino acids (arginine, lysine, and cysteine), leading to the formation of AGEs (Martinez-Saez et al., 2019). These AGE precursors are known to be the cause of many chronic diseases, such as T2DM, Alzheimer's, several types of cancer, and diabetes complications. It is important in terms of health to reduce these products. Some nutrients, such as polyphenols, vitamins, catechins, and proanthocyanidins, inhibit the occurrence of GO and MGO in foods (Wu & Yen, 2005; Peng et al., 2010). It is believed that adding or enriching food with these functional compounds may prevent the formation of DCs in both the food processing stage and in the digestion stage.

4 Conclusion

There are limited studies in the literature on the *in vitro* formation of GO and MGO. Those conducted using selected foods have shown that these precursors are reduced *in vitro*, which leads to the formation of AGEs; however, our *in vitro* study showed that the GO and MGO content generally increased by >100% of the initial value in the processed meat products. The high fat-containing foods may cause lipid oxidation; consequently, α -DCs can be formed. As we observed in the present study, in addition to fat content, nugget and meatball samples contained carbohydrates, and it is believed that the carbohydrates in the meat samples may have contributed to the amount of GO and MGO formed. Thus, reducing saturated fat, carbohydrate, and salt content in foods may result in low levels of GO and MGO during food processing or digestion. Additional studies are needed to support these results.

Conflict of interest

The authors declare no conflict of interest.

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