BIOACCESSIBILITY OF α -DICARBONYL COMPOUNDS IN BREADS AND OTHER BAKERY PRODUCTS USING AN *IN VITRO* STIMULATED GASTROINTESTINAL DIGESTIVE SYSTEMS

M. YAMAN†, E. EDE-CINTESUN†, M. OZGOLET‡, E. SERDAR†,
O.F. MIZRAK† and E. YILDIRIM-SERVİ§

† *Department of Nutrition and Dietetics, Istanbul Sabahattin Zaim University, Istanbul, 34303, Turkey.*
mustafa.yaman@izu.edu.tr
‡ *Department of Food Engineering, Yildiz Technical Universiy, Istanbul, Turkey.*
muhammed.ozgolet@gmail.com
§ *Halal Foods R& D Center, Istanbul Sabahattin Zaim University, Istanbul, 34303, Turkey*
esra.servi@izu.edu.tr

***Abstract*— Digestive system conditions may increase or decrease the formation α-dicarbonyl compounds. This study aims to determine the bioaccessibility of glyoxal (GO) and methylglyoxal (MGO) in bakery products under *in vitro* gastrointestinal conditions. For this purpose breads and bakery products were obtained from bakehouse in Istanbul, Turkey. The amount of GO and MGO in samples ranged between 54.8 to 180.4 µg /100 g and 45.8 to 220.3 µg / 100g respectively. After *in vitro* digestion GO and MGO levels were increased to between 170.4 to 656.8 µg / 100g and 105.6 to 1220.9 µg/100 g, respectively. Bioaccessibility of GO and MGO increased up to 727% and 1351% respectively. High increase rates were observed in samples that contain high fat and low moisture contents. We demonstrated that formation of GO and MGO increase under *in vitro* digestive system conditions. Further studies are needed to extensively investigate the formation mechanism in such foods.**

***Keywords*— Breads, Glyoxal, Methylglyoxal, Bioaccesibility, HPLC.**

I. INTRODUCTION

Bread, with many different forms, is one of the most consumed staple food in the human's diet over the centuries (Cauvain, 2015). Breadmaking requires 3 steps including dough mixing, fermentation, and baking which results unique aroma, taste, and flavor features to bread (Cho and Peterson, 2010). This is a complex process where significant physical and chemical changes occur (Serpen *et al*., 2012). One of the most outstanding chemical changes take place during bread baking is the Maillard reaction (MR). MR is a heat-induced, non-enzymatic browning reaction which occurs between carbonyl groups of reducing sugars and amino groups of amino acids (Helou *et al*., 2016). Hodge first described MR in three stage: initial, intermediate, and final stages where MR products formed. At the initial stage of MR, the Schiff base is formed as a result of the condensation reaction between the carbonyl group of the reducing sugars and the free amino groups of the proteins. During the intermediate stage, the Schiff base converts to ketoamines known as Amadori products. In the final stage, aldol condensation occurs thus brown macromolecules known as melanoidins are generated (Tamanna and Mahmood, 2015). Apart from these series of reactions, MR can take place in biological systems, and the final products are referred as Advanced Glycation End Products (AGEs). AGEs are a heterogeneous group of molecules formed as a result of non-enzymatically by the reaction of reducing sugar and other α-dicarbonyl compounds (α-DCs) with amino groups on proteins, lipids, and nucleic acids. α-DCs, such as 1-deoxyglucose, 3-deoxyglucose (3-DGO), glyoxal (GO), and methylglyoxal (MGO), are AGEs precursors and can be formed as a result of the degradation of Amadori products through MR. α-DCs, form advanced glycation end products (AGEs) in the 'advanced' processes of the MR. As well as MR AGEs can also be derived from caramelization, sugar autoxidation, and lipid peroxidation. The pathway through which AGEs are produced depends on food composition and processing conditions. Caramelization occurs over 120 ℃ or pH between 3-9 whereas Maillard reaction requires 50 ℃ and pH in range of 4-7. Sugar autoxidation takes place under all conditions that are relevant for food processing, especially in alkaline pH. Lipid peroxidation can be formed due to storage and process conditions. Carboxymethyllizine (CML), carboxyethyllizine (CEL), methyl glyoxal lysine dimer (MOLD), deoxyglucose lysine dimer (GOLD), pentosidine, and pyrimidine are the known AGEs (Wang, 2019). Excessive amounts of AGEs in circulation disrupt cell structure and functions which causes oxidative stress and inflammation. AGEs play direct role in the development of age-related chronic degenerative diseases, diabetes mellitus, cardiovascular diseases, cancer and central nervous system disorders (Luevano-Contreras and Chapman-Novakofski, 2010). From this point of view, it is important to know AGEs formation in vitro conditions. Recent studies have focused on the in vitro formation of 'thermal process contaminants' such as α-DCs, AGEs, and lipid oxidation products (Hamzalıoğlu and Gökmen, 2020). Especially lipid oxidation products have been recently studying with great attention. Lipid oxidation degrades food quality, alters texture properties and reduces shelf life in foods as well as increases the

risk of atherosclerosis, age-related chronic conditions, cancer, and neurodegenerative disease by causing inflammation in human body (Guéraud *et al*., 2010). Additionally, oxidation process of lipids increases under gastric digestion due to pro-oxidant conditions in the gastrointestinal tract such as low pH of gastric juice, presence of oxygen incorporated into food during mastication, and metallic ions. The increased lipid oxidation products may also promote the formation of α-DCs (Nieva-Echevarría *et al*., 2020). The effects of digestion on α-DCs occurring in food have not been extensively investigated. In vitro simulated digestion can reduce or strongly increase free α-DCs content depend on food content but or reactions occurring during digestion (Papetti *et al*., 2014). To our knowledge, although the effect of gastric conditions on α-DCs have been studied with some foods including Manuka honey, biscuits, coffee, and soya sauce, there is no study on bread and other bakery products. This study aims to investigate the formation of α-DCs in bakery products under in vitro gastrointestinal system conditions.

II. METHODS

A. Materials

Glyoxal, methylglyoxal, methanol, sodium acetate, 4-nitro-1,2-phenylenediamine, acetonitrile, pancreatin (8 ×USP), pepsine (≥250 units/mg solid), lipase (100-500 units/mg protein), alpha-amylase (1.5 U/mg), NaCl, KCl, bovine serum albumin, bile salts, mucin, urea were procured from Sigma-Aldrich (St. Louis, MO, USA).

B. Sampling

Breads (10), bagels (3), breadsticks(5) and other bread-like product with different additives such as tahini, fruits and some nuts (5) were purchased from different markets in Istanbul, Turkey. The contents of the bakery products were given in Table 1.

C. Extraction and derivatization of GO and MGO in foods

GO and MGO in food was extracted by the method described by Cengiz *et al.* (2020). Following all samples was homogenized with a blender, 5 g of each sample was placed into 50 mL plastic centrifuge tubes and 5 ml methanol was added. Each sample was homogenized by Ultra-Turrax homogenizer and centrifuged at 8000 rpm for 5 min. The supernatant (0.5 ml) was poured into 10 mL glass tube and pH was adjusted to 3 with 0,1 M sodium acetate buffer. Derivatization solution was prepared solving 4 - nitro- 1,2 - phenylenediamine in methanol (1%) and added to each sample mixture. The mixture was allowed to form stable nitroquinoxaline compounds at 70°C for 20 min. The cellulose acetate filter (CA, 0.45 µm) was utilized to filter the samples before injection into HPLC.

D. HPLC determination of GO and MGO

The procedure described by Cengiz *et al.* (2020) was followed to detect GO and GO of the samples via HPLC. The HPLC system includes Shimadzu LC 20 AT pump with a Shimadzu SPD-20A UV/VIS detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase was the solution of methanol: water: acetonitrile (42:56:2, v/v/v). Inertsil ODS-3, 250 x 4.6 mm, 5 µm column was used to separate nitroquinoxaline compounds as a result of the reaction between 4-NPD and, GO and MGO. The flow rate and column temperature were set 1 ml/min and 30 °C. The wavelength of the detector was adjusted to 254 nm.

E. In vitro bioaccesibility of GO and MGO

The bioaccessibility of the GO and MGO in bakery products was determined using an in vitro simulated human digestive system. This in vitro simulated method was a modified version of that described previously by Yaman and Mızrak (2019). Saliva, gastric, duodenal, and bile juices were prepared as 1000 mL and shown in Fig. 1.

For sample digestion, 5 g of bread samples were initially blended with 5 mL of saliva solution. The mixtures were incubated for 5 min at 37 °C. Next, the samples were mixed with 10 mL of gastric solution and allowed for digestion for 30 min at 37 °C. Following gastric phase, the sample mixtures were blended with 5 mL of bile juice and 10 mL of duodenal solution and incubated for 2 h at 37oC. The incubations were carried out in a shaking bath in all phases. After digestion was

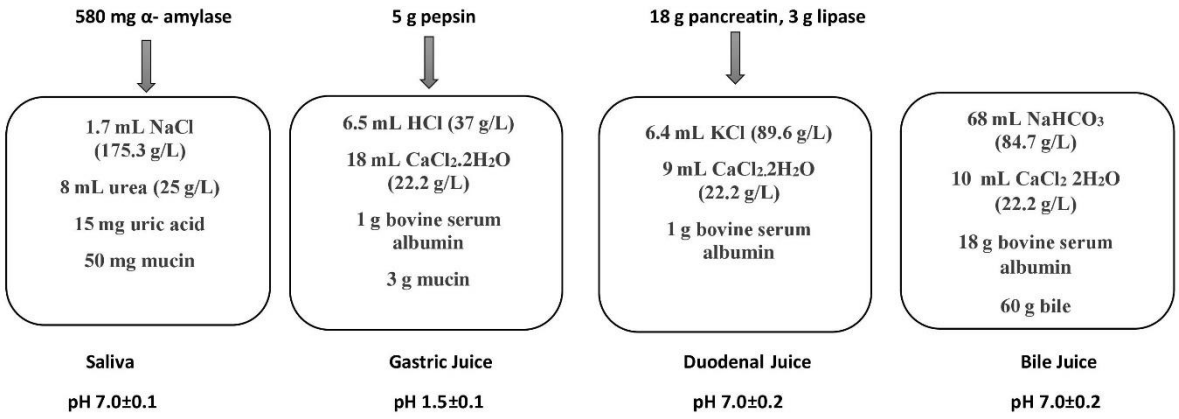

Figure 1. Saliva, gastric, duodenal, and bile juices used in the in vitro human digestion model.
Table 1. The declared amount of macronutrients in breads and other bakery products.

Sample number	Sample type	Energy (kcal/100 g)	Carbohydrate (g/100 g)	Sugar (g/100 g)	Fat (g/100 g)	Saturated Fat (g/100 g)	Protein (g/100 g)
1	Mediterranean bread, plain	283	55.15	2.92	2.81	0.36	9.18
2	Mediterranean bread, wholemeal	264	46.61	2.78	2.87	0.36	11.06
3	Bread, sourdough, wholewheat	214	51	0.86	0.84	0.54	10.7
4	Bread, wholewheat	229	40.6	2.5	1.4	0.3	10.7
5	Toast bread, light	21.24	48.98	1.71	1.42	0.86	11.51
6	Village bread	250	49.44	2.86	1.11	0.22	8.15
7	whole wheat bread, chia seeds	243	40.2	3.9	2.2	1.5	12.2
8	Bread, rye and buckwheat	234	42.8	4.1	1	0.6	11.4
9	Bread, germ	235.93	50.23	2.65	1.03	0.54	9.69
10	Bread, walnut	242	46.5	2.3	2.4	0.45	8.5
11	Breadstick, sesame	415	69.5	4.5	10	5.4	11.8
12	Breadstick, wholemeal	390	75	3	4.7	6	11.3
13	Breadstick, chocolate, butter, milk	487.9	77	6.6	14.3	5.5	12.8
14	Breadstick, oats and buckwheat	420	63	3.11	11.3	3.24	13.4
15	Golden muffin, nut and dried grape	303	46.46	6.98	8.25	2.24	8.95
16	Hard biscuit, sunflower seed	390	28.64	1.3	4.79	2.12	10.21
17	Cracker stick, multigrain	437	65.7	2.88	13.3	4.47	11.5
18	Turkish bagel, sesame	270	53.02	5.3	1.7	0.4	10.52
19	Soft Turkish bagel	289	55.73	12.13	3.5	1.4	8.11
20	Pastry, cheese,dill	340	32.11	1.48	19.85	8.82	8.39
21	Muffin, sweet	372	50.9	31.73	16.4	3.07	6.2
22	Muffin roll, tahini	448	37.92	1.31	29.08	4.4	13.24
23	Flat bread, tahini	471.6	51.4	1.6	24.4	3.2	11.6

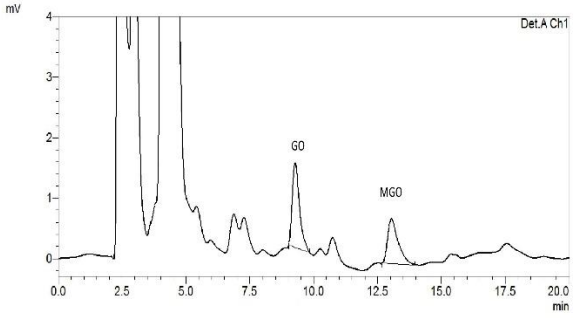

Figure 2. HPLC chromatogram of glyoxal (GO) and methylglyoxal (MGO) in sample 16.

ended, the volume of the digested sample solutions was completed to 50 mL. These solutions were centrifugated for 5 min at 8000 rpm and lastly, the supernatants were filtered with 0,45 µm CA filter before injection to HPLC.

F. Statistical analysis

The results were obtained from three replicates of analysis and given as mean value ± standard deviation. Significant differences was demonstrated by the analysis of variance (ANOVA) and multiple comparison test by Minitab 17 statistical programme ($p<0,05$, Tukey's test).

III. RESULTS & DISCUSSION

The HPLC chromatogram of GO and MGO in sample 16 are shown in F. 2. The amount of macronutrients on the labels including energy, carbohydrate, fiber, sugar, fat, protein, and salt were presented in Table 1. Table 1 shows that the bread samples (sample 1-10) contain high amount of carbohydrate ranged from 40.2 to 55.15 g/100 g, whereas breadsticks and cracker sticks (sample 11-18) have high carbohydrate and fat content ranged from 28.64 to 77 g/100g and 4.7 to 13.3 g/100 g, respectively. Similarly, other bread-like bakery products (sample 19-23) include significant carbohydrate and fat ranged from 37.9 to 55.7 g/100 g and 1.7 to 29.08 g/100 g respectively.

The GO and MGO levels before and after digestion were given in Table 2. The amount of GO and MGO in samples ranged between 54.8 to 180.4 µg/100 g and 45.8 to 220.3 µg/100 g respectively at initial. In accordance with our findings, Degen *et al*. (2012) detected MGO content with average value of 3.0 mg/kg; Uribari *et al*. (2010) found 3.6 nmol/100 g MGO in bread samples; Maasen *et al*. (2020) indicated α-DCs in breads were ranged 90 to 146 mg/kg. α-DCs in breads can be easily formed by sugars in caramelization reactions, during the Maillard reaction, or lipid peroxidation (Wang 2019). Among the samples, 16, 18, 19, 22 and 23 had the highest GO and MGO content at initial. Sample 18, 22 and 23 contain sesame or tahini. Tahini and sesame are rich sources of unsaturated fatty acids (Elleuch *et al.,* 2011). This fat content may be contributed to α-DCs due to lipid peroxidation. Along with these findings, it is important to know α-DCs formation under in vitro gastrointestinal digestion conditions. MR products, lipid peroxidation products, AGEs, and α-DCs have also been produced endogenously under digestive system (Aljahdali and Carbonero, 2019).

GO and MGO amounts of the samples after in vitro gastrointestinal system were displayed in Table 2. The amount of GO and MGO in breads in vitro gastrointestinal after digestion were ranged from 170.4 to 656.8 µg/100g and 105.6 to 1220.9 µg/100 g, respectively. Our results revealed that α-DCs were increased in bread under in vitro digestive system. The increase of GO and MGO following digestion in breads was shown in Fig. 3.

Table 2. GO and MGO contents of samples at initial and *in vitro* digestion and increase rate.

Sample number	Sample type	Initial value GO (µg/100 g)	After Digestion GO (µg/100 g)	Initial value MGO (µg/100 g)	After Digestion MGO (µg/100 g)	Bioaccessibility % GO	Bioaccessibility % MGO
1	Mediterranean bread	71.8±2.5^a	221.3±7.8^b	69.8±2.5^a	193.4±6.8^b	309.6±11.0	278.3±9.9
2	Mediterranean bread, wholemeal	72.8±2.6^a	192.4±6.8^b	101.7±3.6^a	200.3±7.1^b	265.5±9.4	197.9±7.0
3	Bread, sourdough, wholewheat	72.8±2.6^a	211.3±7.4^b	71.8±2.5^a	194.4±6.8^b	291.6±10.3	272.0±9.6
4	Bread, wholewheat	63.8±2.2^a	174.4±6.1^b	62.8±2.2^a	129.6±4.6^b	274.6±9.7	207.2±7.3
5	Toast bread, light	59.8±2.1^a	176.4±6.2^b	61.8±2.2^a	156.5±5.5^b	296.2±10.5	254.3±9.0
6	Village bread	64.8±2.3^a	206.3±7.3^b	75.7±2.7^a	166.4±5.9^b	319.8±11.3	220.7±7.8
7	whole wheat bread, chia seeds	82.7±2.9^a	204.3±7.2^b	45.8±1.6^a	128.6±4.5^b	248.0±8.8	281.6±10.0
8	Bread, rye and buckwheat	87.7±3.1^a	219.3±7.7^b	51.8±1.8^a	131.6±4.6^b	251.0±8.9	254.9±9.0
9	Bread, germ	65.8±2.3^a	170.4±6.0^b	86.7±3.1^a	181.4±6.4^b	260.2±9.2	210.1±7.4
10	Bread, walnut	54.8±1.9^a	200.3±7.1^b	121.6±4.3^a	181.4±6.4^b	367.0±13.0	149.8±5.3
11	Breadstick, sesame	71.8±2.5^a	490.4±17.3^b	86.7±3.1^a	615.9±21.7^b	686.2±24.3	713.3±25.3
12	Breadstick, wholemeal	74.8±2.6^a	567.1±20.0^b	90.7±3.2^a	1220.9±43.0^b	761.8±27.0	1351.8±47.9
13	Breadstick, chocolate, butter, milk	90.7±3.2^a	656.8±23.1^b	133.6±4.7^a	992.7±35.0^b	727.2±25.8	746.4±26.4
14	Breadstick, oats and buckwheat	65.8±2.3^a	302.0±10.6^b	85.7±3.0^a	190.4±6.7^b	461.0±16.3	223.0±7.9
15	Golden bread, nut and dried grape	87.7±3.1^a	249.2±8.8^b	72.8±2.6^a	105.6±3.7^b	285.3±10.1	145.8±5.2
16	Hard biscuit, sunflower seed	109.6±3.9^a	518.3±18.3^b	158.5±5.6^a	396.7±14.0^b	474.7±16.8	251.4±8.9
17	Cracker stick, multigrain	70.8±2.5^a	345.8±12.2^b	76.7±2.7^a	299.0±10.5^b	490.8±17.4	391.2±13.9
18	Cracker stick, multigrain	116.6±4.1^a	372.8±13.1^b	220.3±7.8^a	382.7±13.5^b	321.0±11.4	174.5±6.2
19	Turkish bagel, sesame	114.6±4.0^a	306.0±10.8^b	194.4±6.8^a	202.3±7.1^a	268.1±9.5	104.5±3.7
20	Soft Turkish bagel	74.8±2.6^a	192.4±6.8^b	153.5±5.4^a	209.3±7.4^b	258.4±9.2	136.9±4.9
21	Pastry, cheese,dill	70.8±2.5^a	225.2±7.9^b	97.7±3.4^a	209.3±7.4^a	319.6±11.3	215.2±7.6
22	Muffin, sweet	180.4±6.4^a	328.9±11.6^b	203.3±7.2^a	286.0±10.1^a	183.1±6.5	141.3±5.0
23	Muffin roll, tahini	123.6±4.4^a	231.2±8.1^b	139.5±4.9^a	222.3±7.8^a	187.9±6.7	160.0±5.7

Values are means ± range, n = 3. The different letters in the same rows for GO and MGO show that there are statistical differences (ANOVA $p < 0.05$, Tukey's test.)

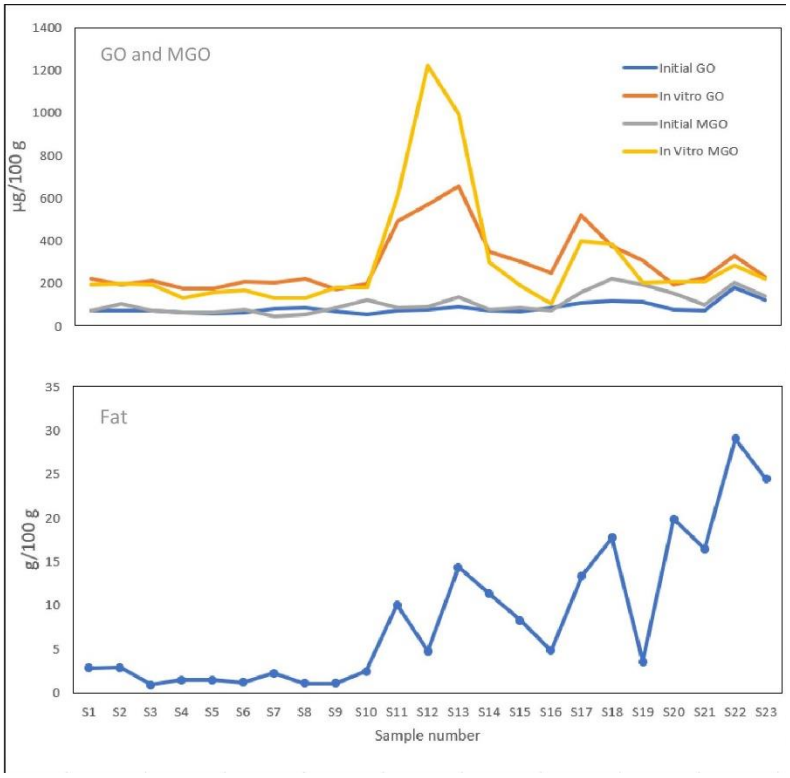

Figure 3. Fat and initial and after in vitro GO and MGO contents of samples.

The formation of α-DCs in the gastrointestinal system may differ depending on food composition or food processing conditions (Papetti *et al.*, 2014). As seen from Table 2, bioaccessibility of GO and MGO in breads (sample 1-10) were increased range from 248 to 367 % and 149 to 281 % respectively. The increase rate of α-DCs were higher in refined flour breads (sample 1 and sample) whereas lower in whole grain breads (sample 7 and sample 8) which may be related to the antioxidant capacity of whole grains. Whole grains such as buckwheat, rye, chia seeds have high nutritional value due to their balanced amino acid composition and high contents of vitamins

and proteins. They are also rich in antioxidant compounds due to their phenolic compounds (Jiang *et al*., 2007). Since GO and MGO level increases with oxidative stress, antioxidants may prevent GO and MGO formation (Fu *et al*., 1994). Szawara-Nowak *et al.* (2014) evaluated *in vitro* inhibitory effects of buckwheat enhanced wheatbread extracts on the formation of AGEs. The extracts from buckwheat enhanced dark wheat breads showed the highest inhibitory activity against AGEs and the inhibitory effect was attributed to phenolic compounds derived from buckwheat addition. Similarly, Gawlik-Diziki *et al*. (2009) investigated the impact of *in vitro* digestion on bioactive characteristics of wheat bread with Tartary buckwheat flavones addition. Their results showed that free radical scavenging activity of Tartary buckwheat flavones decreases lipid peroxidation during digestion. Mildner-Szkudlarz *et al*. (2017) have also studied the effects of phenolic compounds including catechin, quercetin, gallic, ferulic, and caffeic acids on reducing formation of Ne-(carboxymethyl) lysine and pyrazines formed by Maillard reactions in a model bread system. They showed that phenolic compounds inhibit AGEs formation, among phenolic compounds ferulic acid was the strongest inhibitor effect up to 62 %. Kocadağlı *et al*. (2016) also observed the quantities of GO and MGO were negatively correlated with total phenolic compounds in biscuits following baking. In addition to this, another remarkable finding is the effects of walnut bread on α-DCs formation. The bioaccessibility of GO increased 367 % while MGO 149 % in Sample 10. Glyoxal can contribute AGEs formation mostly through lipid peroxidation whereas MGO can through fructose catabolism (Singh *et al*., 2001). These results may be attributed to fat content which walnut increased in breads. Due to fat content *in vitro* GO formation was higher than MGO in walnut bread. The bioaccessibility of GO and MGO were higher in breads (sample 1-10) than breadsticks and cracker sticks (sample 11-18). This result may be related to fat content, moisture, sugar content, food processing, and heat treatment conditions. Nutrient composition and food processing conditions may also effect *in vitro* formation of α-DCs (Papetti *et al.*, 2014).

The amount of GO and MGO in breadsticks and cracker sticks increased 285.3 to 761.5 % and 145.8 to 1351.8 % under *in vitro* conditions. As seen from Figure 3, The increase rate of GO and MGO were correlatively with fat and saturated fat content. Among breadsticks, sample 12 had the highest α-DCs content and fat content. Since α-DCs can be formed through lipid peroxidation this increase was highly reasonable. Different factors influence lipid peroxidation rate such as heat, light, reactive oxygen species, metal ions and fat composition. Polyunsaturated fatty acids have more tendency to lipid peroxidation than saturated fats. (Barden *et al*., 2015). Also, oxidative degradation of lipids through gastrointestinal tract increases due to the presence of high amount of oxidants (Nieva-Echevarría *et al.*, 2020). Apart from lipid peroxidation, sugar autoxidation, low moisture may contribute to α-DCs formation in breadsticks.

The increase rate in sample 15 may be formed by sugar autoxidation due to dried grape content. Like breads, in breadsticks with buckwheat had lower increase rate, this may be related to antioxidant effects of buckwheat. Sample 13 had high level of GO and MGO. This may be attributed to sugar autoxidation, caramelization, and lipid peroxidation derived from the presence of chocolate, butter, and milk in the formulation. Lipid peroxidation in low moisture foods is serious problem due to their polyunsaturated fat content, however, this issue has been poorly studied (Barden and Decker, 2016). Our results are important in terms of comparing *in vitro* α-DCs formation in breads with low moisture breadsticks.

As seen in Table 2, the GO and MGO content had increased in other bakery products (sample 19-23) 183.1 to 321 % and 104.5 to 215.2 % respectively. The increased rate was similar to bread samples and lower than breadsticks and cracker sticks. The differences between samples may be attributed to moisture content, food processing, heat treatment conditions, and food composition. Among other bakery products, sample 21 had the highest α-DCs values. Due to cheese content, the α-DCs formulation might occur through glycose autoxidation (Nie *et al*., 2020) . Sample 19 and sample 20 had high α-DCs, and these samples were also had high sugar content. Sugar autoxidation might increase *in vitro* GO and MGO formulation in these samples. The increase in sample 22 and 23 may be attributed to tahini content by sugar auto oxidation.

These results showed that bread, breadsticks, cracker sticks, and other bakery products had high GO and MGO content. The high AGEs exposure with ingestion of food may cause AGEs accumulation in body. Excessive AGEs in tissues may cause oxidative stress and inflammation, which contribute to pathogenesis of chronic diseases (Luevano-Contreras and Chapman-Novakofski, 2010). Oxidative stress and inflammation cause the production of angiotensin II, which contribute the pathogenesis of gastrointestinal disease, especially liver disease (Ahmadian *et al*., 2016). Also, AGEs can play a direct role in the risk of developing diabetes, cardiovascular diseases, central nervous system disorders, and even cancer. Several studies point out that the presence of AGEs in human tissues may cause tumor growth via protein damage, aberrant cell signaling, increased stress responses, and decreased genetic fidelity. AGEs receptors can cause cancer by activating the interleukin mediated mitochondrial signal transducers and activators of transcription 3 (STAT3) signaling in pancreatic carcinogenesis.

To limit AGEs exposure, it is important to reduce AGEs formation in foods (Kang *et al*., 2012). Adding antioxidants to foods is practical strategy used to reduce AGEs formation. Antioxidants can inhibit or delay oxidation by scavenging free radicals and preventing radical chain reactions. In this context, adding antioxidants to bread and bakery products can be used in new formulations (Ede-Çintesun *et al*., 2022a,b).

The formation of α-DCs may occur due to fat content, food processing techniques, heat treatment, and moisture

level through lipid peroxidation, sugar autooxidation, caramelization, and MR (Vistoli *et al*., 2013). Also, α-DCs formation increases under *in vitro* digestion in breads, breadsticks, cracker sticks and other bakery products. α-DCs increase in parallel with fat content especially in breadsticks and cracker sticks. This reveals the importance of α-DCs formation through lipid peroxidation. It may be considered that GO and MGO can be used as an indicator to evaluate lipid peroxidation during *in vitro* digestion. Different studies showed that α-DCs may increase or decrease *in vitro* gastrointestinal system depending upon food composition or food processing. Since the results in the literature are not consistent, further studies are needed. This study has unique value in terms of indicating increase α-DCs in breads and bread-like products under *in vitro* digestive system conditions.

V. CONCLUSIONS

In conclusion, breads, breadsticks, cracker sticks, and other bakery products contain high levels of GO and MGO due to fat and sugar content, low moisture, and high baking temperatures. α-DCs can be easily formed by MR, sugar autooxidation, and lipid peroxidation. Whole grain breads and bakery products have less α-DCs contents than refined samples. These differences may be attributed to antioxidant compounds that whole grains consist of. Also, GO and MGO levels increase under *in vitro* gastrointestinal system conditions. Especially this increases were high in samples with high fat content and low moisture such as breadsticks and cracker sticks through lipid peroxidation. Lipid peroxidation increases under gastric digestion which may be associated with the presence of pro-oxidants in the gastrointestinal tract due to the factors such as low pH of gastric juice, and metallic ions. Since this study have assumed that GO and MGO in breads increase under *in vitro* conditions, further studies may focus on the pathways of α-DCs formation in such foods.

REFERENCES

Ahmadian, E., Pennefather, P.S., Eftekhari, A., Heidari, R. and Eghbal, M.A. (2016) Role of renin-angiotensin system in liver diseases: an outline on the potential therapeutic points of intervention. Expert. *Rev. Gastroenterol. Hepatol*. **10,** 1279-1288.

Aljahdali, N. and Carbonero, F. (2019) Impact of Maillard reaction products on nutrition and health: Current knowledge and need to understand their fate in the human digestive system. *Crit. Rev. Food Sci. Nutr*. **59**, 474–487

Barden, L., Vollmer, D., Johnson, D. and Decker, E. (2015) Impact of iron, chelators, and free fatty acids on lipid oxidation in low-moisture crackers. *J. Agric. Food Chem*. **63**, 1812–1818.

Barden, L. and Decker, E.A. (2016) Lipid oxidation in low-moisture food: a review. *Crit. Rev. Food Sci. Nutr*. **56**, 2467–2482

Cauvain, S. (2015) Breadmaking processes.in Technology of breadmaking. Springer 23–55.

Cengiz, S., Kişmiroğlu, C., Çebib, N., Çatak, J. and Yaman, M. (2020) Determination of the most potent precursors of advanced glycation end products (AGEs) in chips, crackers, and breakfast cereals by high performance liquid chromatography (HPLC) using precolumn derivatization with 4-nitro-1, 2-phenlenediamine. *Microchem. J*. **158,** 105170.

Cho, I.H. and Peterson, D.G. (2010) Chemistry of bread aroma: A review. *Food Sci. Biotech*. **19**, 575–582.

Degen, J., Hellwig, M. and Henle, T. (2012) 1, 2-Dicarbonyl compounds in commonly consumed foods. *J. Agric. Food Chem*. **60**, 7071–7079.

Ede- Çintesun, E., Tanyıldız, S.N., Yıldırım, H., Mızrak, Ö. F. and Yaman, M. (2022a). Investigation of the alpha-dicarbonyl compounds in some snack foods by HPLC using precolumn derivatization with 4-Nitro-1, 2-Phenylenediamine. *Biointerface Res. Appl. Chem*. **12**, 2242-2250.

Ede-Cintesun, E. Yaman, M., Aslan, R., Mizrak, Ö.F. and Nur, S. (2022b) Effects of Different Herbal Teas on Reducing the Bioaccessibility of Methylglyoxal in Crackers under Stimulated Gastrointestinal Digestive System. *Lett. Appl. Nano Bio Science*. **11**, 3421-3429.

Elleuch, M., Bedigian, D. and Zitoun, A. (2011) Sesame (Sesamum indicum L.) seeds in food, nutrition, and health. *Nuts and seeds in health and disease prevention*. Elsevier 1029–1036.

Fu, M.-X., Wells-Knecht, K.J., Blackledge, J.A., Lyons, T.J., Thorpe, S.R. and Baynes, J.W. (1994) Glycation, glycoxidation, and cross-linking of collagen by glucose: kinetics, mechanisms, and inhibition of late stages of the Maillard reaction. *Diabetes*. **43**, 676–683

Gawlik-Dziki, U., Dziki, D., Baraniak, B. and Lin, R. (2009) The effect of simulated digestion in vitro on bioactivity of wheat bread with Tartary buckwheat flavones addition. *LWT-Food Sci. Technol*. **42,** 137–143

Guéraud, F., Atalay, M., Bresgen, N., Cipak, A., Eckl, P.M., Huc, L., Jouanin, I., Siems, W. and Uchida, K. (2010) Chemistry and biochemistry of lipid peroxidation products. *Free Radic. Res*. **44,** 1098–1124

Hamzalıoğlu, A. and Gökmen, V. (2020) Potential reactions of thermal process contaminants during digestion. *Trends Food Sci. Technol*. **106,** 198-208.

Helou, C., Jacolot, P., Niquet-Léridon, C., Gadonna-Widehem, P. and Tessier, F.J. (2016) Maillard reaction products in bread: A novel semi-quantitative method for evaluating melanoidins in bread. *Food Chem*. **190,** 904–911

Jiang, P., Burczynski, F., Campbell, C., Pierce, G., Austria, J.A. and Briggs, C.J. (2007) Rutin and flavonoid contents in three buckwheat species Fagopyrum esculentum, F. tataricum, and F. homotropicum and their protective effects against lipid peroxidation. *Food Res. Int*. **40,** 356–364.

Kang, R., Loux, T., Tang, D., Schapiro, N. E., Vernon, P., Livesey, K.M., Krasinskas, A., Lotze, M.T. and Zeh, H.J. (2012) The expression of the receptor for advanced glycation endproducts (RAGE) is permissive for early pancreatic neoplasia. *Proc. Natl. Acad. Sci.* **109**, 7031-7036.

Kocadağlı, T., Žilić, S., Taş, N.G., Vančetović, J., Dodig, D. and Gökmen, V. (2016) Formation of α-dicarbonyl compounds in cookies made from wheat, hull-less barley and colored corn and its relation with phenolic compounds, free amino acids and sugars. *Eur. Food Res. Tech*. **242,** 51–60

Luevano-Contreras, C. and Chapman-Novakofski, K. (2010) Dietary advanced glycation end products and aging. *Nutrients*. **2,** 1247–1265

Maasen, K. et al. (2020) Quantification of dicarbonyl compounds in commonly consumed foods and drinks; presentation of a food composition database for dicarbonyls. Food Chem. **339,** 128063

Mildner-Szkudlarz, S., Scheijen, J.L.J.M., Opperhuizen, A., Stehouwer, C.D.A., Van Greevenbroek, M.M. and Schalkwijk, C.G. (2017) Phenolic compounds reduce formation of Nε-(carboxymethyl) lysine and pyrazines formed by Maillard reactions in a model bread system. *Food chem*. **231,** 175–184

Nie, C., Li, Y., Qian, H., Ying, H. and Wang, L. (2020) Advanced glycation end products in food and their effects on intestinal tract. *Crit. Rev. Food Sci. Nutr*. **22**, 3103-3115.

Nieva-Echevarría, B., Goicoechea, E. and Guillén, M.D. (2020) Food lipid oxidation under gastrointestinal digestion conditions: A review. *Crit. Rev. Food Sci. Nutr.* **60,** 461–478

Papetti, A., Mascherpa, D. and Gazzani, G. (2014) Free α-dicarbonyl compounds in coffee, barley coffee and soy sauce and effects of in vitro digestion. *Food Chem*. **164,** 259–265

Serpen, A., Gökmen, V. and Mogol, B.A. (2012) Effects of different grain mixtures on Maillard reaction products and total antioxidant capacities of breads. *J Food Compost. Anal*. **26,** 160–168

Singh, R., Barden, A., Mori, T. and Beilin, L. (2001) Advanced glycation end-products: a review. *Diabetologia* **44,** 129–146

Szawara-Nowak, D., Koutsidis, G., Wiczkowski, W. and Zieliński, W. (2014) Evaluation of the in vitro inhibitory effects of buckwheat enhanced wheat bread extracts on the formation of advanced glycation end-products (AGEs). *LWT-Food Sci. Technol*. **58,** 327–334

Tamanna, N. and Mahmood, N. (2015) Food processing and maillard reaction products: effect on human health and nutrition. *Int. J Food Sci*. **2015,** 526762.

Uribarri, J., Woodruff, S., Goodman, S., Cai, W., Chen, X., Pyzik, R., Yong, A., Sriker, G.E. and Vlassara, H. (2010) Advanced glycation end products in foods and a practical guide to their reduction in the diet. *J Am. Diet. Assoc*. **110,** 911–916

Vistoli, G., De Maddis,D., Cipak, A., Zarkovic, N., Carini, M. and Aldini, G. (2013) Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. *Free Radic. Res*. **47,** 3–27

Wang, S. (2019) *Chemical Hazards in Thermally-Processed Foods*. Springer.

Yaman, M. and Mızrak, Ö.F. (2019) Determination and evaluation of in vitro bioaccessibility of the pyridoxal, pyridoxine, and pyridoxamine forms of vitamin B6 in cereal-based baby foods. *Food Chem*. **298,** 125042

Received: March 8, 2022
Sent to Subject Editor: April 7, 2022
Accepted: June 2, 2022
Recommended by Subject Editor Laura Briand