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Determination and assessment of the most potent precursors of advanced glycation end products in baklava and Turkish delight by HPLC

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Abstract

This study aims to investigate the glyoxal (GO), and methylglyoxal (MGO), and the effect of the types of sugar components on GO and MGO formation in Turkish delight and baklava. The values of GO and MGO ranged from 326 to 1842 µg/100 g, and from 31 to 517 µg/100 g in Turkish delight, respectively. The values of GO and MGO in baklava ranged from 344 to 815 µg/100 g, and between 19 and 358 µg/100 g, respectively. One of the baklava samples may contained high fructose corn syrup (HCFS) given the presence of significantly higher amount of MGO than that of other samples. Increased sugar concentration, processing time, storage, and HCFS in Turkish delight and baklava may affect the GO and MGO formations. Longer storage time may influence the increase of MGO formation in Turkish delight. Therefore, these products should be consumed less or formulated with agents that reduce AGEs.

Keywords: Maillard reaction; caramelization; AGEs; baklava; Turkish delight.

Practical Application: In this study we observed that, increased sugar concentration, processing time, storage, and in Turkish delight and baklava may affect the glyoxal and methylglyoxal formations. These precursors lead to harmful AGEs as an end result. The increased level of AGEs in human body may cause many diseases. Therefore, both baklava and Turkish delight should be consumed less or formulated with micronutrients that reduce AGEs.

1 Introduction

The consumption of processed foods is gradually increasing in today's modern diet. Maillard reaction products (MR), which increase the sensory properties of foods, are formed during the processing of foods (Uribarri et al., 2010; Somoza, 2005). The MR is initiated by non-enzymatic reactions between reducing sugars and protein amino groups to form an unstable Schiff base, which usually occurs during heat treatment and long-term storage. These unstable Schiff bases can turn into more stably configured products such as Amadori or Heyns. Depending on the reaction time, increase in cooking temperature, and long-term storage conditions, these products lead to the formation of more stable intermediate alpha dicarbonyl compounds (α-DCs) (Wang, 2019; Sharma et al., 2015). Glyoxal (GO) and methylglyoxal (MGO) are the most studied Amadori and Heyns products. GO and MGO react with amino groups of proteins leading to the formation of harmful AGEs (Somoza, 2005). In addition to the MRs, GO and MGO can be produced from sugar autoxidation, lipid oxidation, and microbial fermentation (Amoroso et al., 2013). These products can be also formed through the oxidation of glucose and lipids in human metabolism (Uribarri et al., 2010). Generally, α-DCs compounds can be formed in highly contained polyunsaturated lipid-rich and monosaccharide-rich foods such as cookies, beverages, smoked goods, and some other baked foods (Papetti et al., 2014). The accumulation of α-DCs in the body causes many health problems. These compounds react with amino groups of peptides, amino acids, and proteins to form final AGEs (Sharma et al., 2015). These α-DCs generally react with the amino group of lysine, the thiol group of cysteine, and the guanidine group of arginine. The highly reactive AGEs such as N-ε-carboxymethyllysine (CML) and N-ε-carboxyethyllysine (CEL) are formed by the reaction of GO and MGO with the lysine group of amino acids, respectively (Henle, 2005; Sharma et al., 2015). Many clinical studies reported that the generation of AGEs in the human body can cause chronic and degenerative diseases such as diabetes mellitus, renal failure, Alzheimer's, and Parkinson's diseases. The glucose concentration is high in diabetic patients due to the low levels of insulin secretion from the pancreas; for this reason, increased glucose reacts with body proteins two or three times more than in healthy people, generating highly reactive GO and MGO (Nowotny et al., 2015). The final AGEs such as CEL and CML in processed foods can be formed during long periods of storage. However, forming CEL and CML from reducing sugars and proteins reduces the nutrition quality of foods (Uribarri et al., 2010; Yu et al., 2016). Processed foods such as cereals, bakery products, meat, and dairy contain a significant level of CML, while biscuits, bread

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crust, and pork meat contain CEL (Wang, 2019; Sun et al., 2015). The accumulation of AGEs occurs mostly in collagen and eye proteins, which are long-lived proteins. TAs a result, cataract formation in diabetic patients is the most common problem caused by to the low half-life of these proteins (Nowotny et al., 2015).

Today, in addition to processed foods, the consumption of industrially produced traditional foods is increasing. According to Turkomp (2020), most Turkish traditional foods are rich in carbohydrates. High carbohydrate foods increase blood glucose levels (Thomas et al., 1991; Yaman et al., 2019; Çatak, 2019), and high glucose concentration can cause the formation of AGEs in blood circulation (Nowotny et al., 2015). The purpose of this study was to determine GO, MGO, and free sugar levels in Turkish delight and baklava, and to investigate the effect of the type of sugar components on the formation of GO and MGO.

2 Materials and methods

2.1 Materials

Glyoxal, methylglyoxal, methanol, sodium acetate, 4-nitro-1,2-phenylenediamine, acetonitrile, fructose, glucose, and sucrose were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Sampling

In this research, two different unpackaged Turkish traditional foods (baklava and Turkish delight) were obtained from different markets in Istanbul, Turkey.

2.3 Extraction and derivatization of GO and MGO

The extraction method for GO and MGO in foods described by Cengiz et al. (2020) was used with some modifications. First, 5 g of each sample was weighed into a 50 mL plastic falcon tube and 20 mL methanol was added. Next, the sample was extracted with an ultra-thorax homogenizer for 2 min and centrifuged at 8000 rpm for 5 min. Afterward 0.5 mL of centrifuged liquid sample was taken into a 10 mL glass tube and 1 mL sodium acetate buffer (0.1 M, pH: 3) was added. Then, 0.5 mL of derivatization solution (4-nitro-1,2-phenylenediamine in 1% methanol) was added. The mixture was incubated at 70 °C for 20 min. The samples were filtered using a 0.45 μm cellulose acetate filter and injected into the HPLC.

2.4 Extraction of sugars

The extraction method of sugars described by Richmond et al. (1981) was used with some modifications. First, 5 g of each homogenized sample was weighed into a 50 mL plastic falcon tube. Then, 20 mL deionized water was added and the sample was extracted using the ultra-thorax homogenizer for 5 min. The final volume was adjusted to 50 mL with deionized water. The mixture was centrifuged at 8000 rpm for 5 min. The samples were filtered with a 0.45 μm cellulose acetate filter and injected into the HPLC.

2.5 HPLC determination of GO and MGO

The HPLC conditions described by Yusufoğlu et al. (2020) were used with some modifications. The HPLC system consisted of a Shimadzu LC 20AT pump with a Shimadzu SPD-20A UV/VIS detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase consists of methanol: water: acetonitrile (42:56:2, v/v/v). The wavelength was 255 nm. The GO and MGO were separated with an Inertsil ODS-3, 250 x 4.6 mm, 5 μm, column with a flow rate of 1 mL/min. The column oven temperature was 30 °C.

2.6 HPLC determination of sugars

The HPLC conditions described by Richmond et al. (1981) were used to determine sugar content. The HPLC system consisted of a LC 20AT pump with a Shimadzu RI-20A refractive detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase was composed of acetonitrile: water (80:20, v/v). Fructose, glucose, and sucrose were separated with an Agilent NH_2, 250 x 4.6 mm, 5 μm column (Santa Clara, CA, USA) with a flow rate of 2 mL/min. The column oven temperature was 30 °C.

3 Result and discussion

HPLC chromatogram of GO and MGO in Turkish delight is shown in Figure 1. The AGE formation process in foods is shown in Figure 2. The GO, MGO, fructose, glucose, sucrose, and total sugar values of Turkish delight are presented in Table 1. The GO and MGO values ranged between 326 and

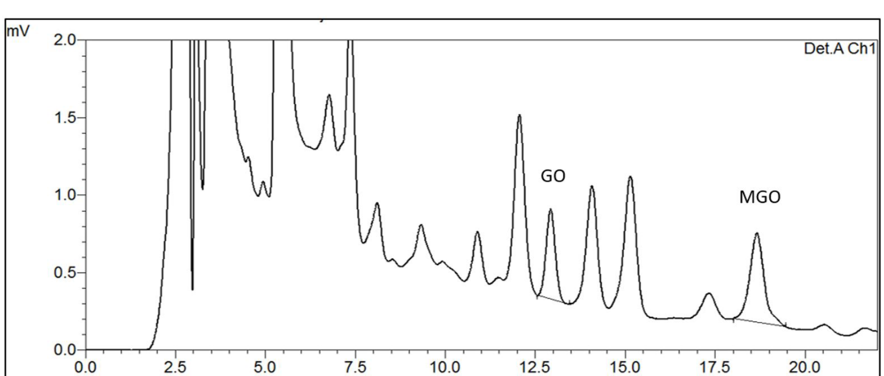

Figure 1. HPLC chromatogram of GO and MGO in Turkish delight.

1842 μg/100 g and from 31 to 517 μg/100 g, respectively, while the fructose, glucose, sucrose, and total sugars ranged from 6.7 to 19.3 g/100 g, 10.5 to 24.2 g/100 g, 7.7 to 28.2 g/100 g, and 26.6 to 63.8 g/100 g, respectively. As can be seen from the results, 3 of the Turkish delight samples had a high amount of MGO (339-517 μg/100 g). These samples also contained high levels of fructose (14.8-19.03 g/100 g) and glucose (16.1-23.7 g/100 g). However, the sucrose values of the samples with high MGO values were found to be generally low. Therefore, it is thought that the amount of sucrose does not affect MGO formation. The GO values of 4 samples were generally higher (1094-1842 μg/100 g) than that of other samples, and both the fructose and glucose levels of these samples were also found to be high in all but except for one sample. Similarly, as in MGO, there is no relevance between sucrose and the GO formation.

Lokum, also known as "Turkish delight", is a traditional food prepared by mixing sugar, water, starch, and citric acid or tartaric acid in a wide container for 2-5 h at 125 °C (Batu & Kirmaci, 2009). According to the Turkomp, the glucose, fructose, sucrose, and total sugar content range between 19.20-20.93 g/100 g, 12.43-21.78 g/100 g, 20.41-24.95 g/100 g, and 58.31-66.3 g/100 g, respectively. The glucose, fructose, sucrose, and total sugar value of 8 out of 14 samples were found to be low compared to Turkomp (2020); the reason may be production techniques or different recipes. As seen from the table, Turkish delight generally contains high amounts of sugar components.

The GO, MGO, and free sugar contents of baklava are given in Table 2. The GO and MGO levels range between 344 and 815 μg/100 g and 19 and 358 μg/100 g, while fructose, glucose,

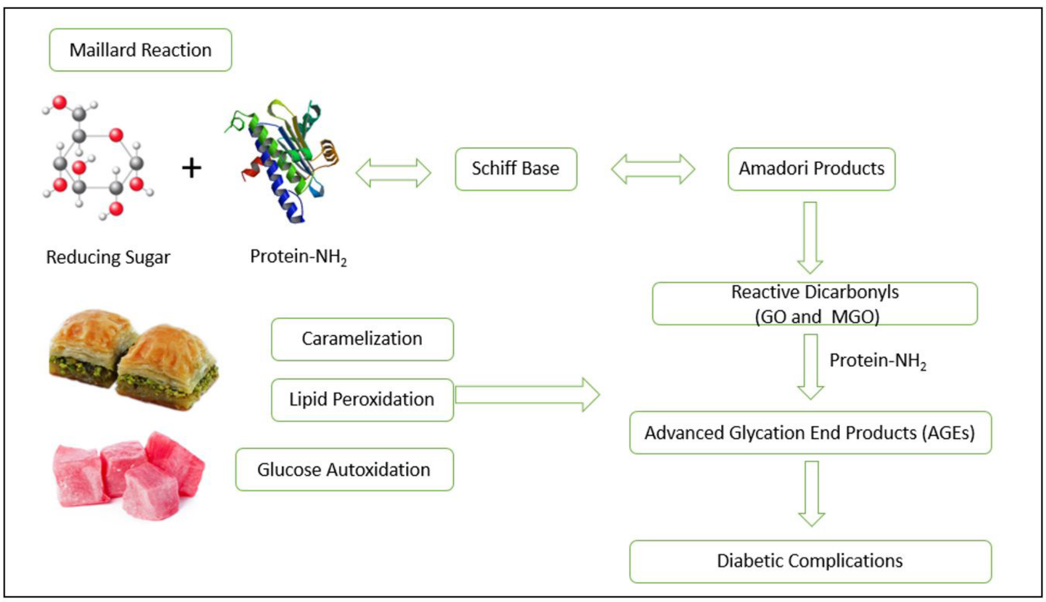

Figure 2. Advanced glycation end products (AGEs) formation process in foods.

Table 1. GO, MGO, and free sugar content in Turkish delight.

Sample	GO μg/100 g	MGO μg/100 g	Fructose g/100 g	Glucose g/100 g	Sucrose g/100 g	Total Sugar g/100 g
T.Delight-1	476 ± 9	227 ± 7	19.3 ± 0.8	24.2 ± 0.3	12.4 ± 0.4	55.9
T.Delight-2	358 ± 6	92 ± 6	9.4 ± 0.4	11.6 ± 0.3	11.6 ± 0.4	32.6
T.Delight-3	326 ± 6	236 ± 7	9.8 ± 0.2	13.1 ± 0.4	9.9 ± 0.4	32.8
T.Delight-4	333 ± 5	183 ± 6	8.1 ± 0.2	10.8 ± 0.3	7.7 ± 0.5	26.6
T.Delight-5	451 ± 7	339 ± 8	19 ± 0.3	23.7 ± 0.3	9.1 ± 0.2	51.8
T.Delight-6	567 ± 9	31 ± 6	10.3 ± 0.3	11.9 ± 0.3	27.6 ± 0.5	49.8
T.Delight-7	827 ± 9	237 ± 7	6.7 ± 0.2	10.5 ± 0.3	19 ± 0.5	36.2
T.Delight-8	396 ± 9	517 ± 6	14.8 ± 0.3	16.1 ± 0.3	20.3 ± 0.3	51.2
T.Delight-9	1094 ± 23	130 ± 7	10.4 ± 0.2	12.3 ± 0.3	24.7 ± 0.7	47.4
T.Delight-10	324 ± 10	133 ± 7	12.3 ± 0.2	14.2 ± 0.2	24.8 ± 0.7	51.2
T.Delight-11	489 ± 9	202 ± 6	11.5 ± 0.2	14.6 ± 0.1	13.9 ± 0.4	40.1
T.Delight-12	1842 ± 15	249 ± 8	19.2 ± 0.3	23.1 ± 0.2	21.5 ± 0.5	63.8
T.Delight-13	1591 ± 14	405 ± 6	15.4 ± 0.3	20.1 ± 0.2	11.1 ± 0.4	46.6
T.Delight-14	1161 ± 20	47 ± 6	13.1 ± 0.3	15.7 ± 0.2	28.2 ± 0.9	57

Values are means ± range, n = 3. GO = glyoxal; MGO = methylglyoxal.

Table 2. GO, MGO, and free sugar content in baklava.

Sample	GO	MGO	Fructose	Glucose	Sucrose	Total Sugar
	μg/100 g	μg/100 g	g/100 g	g/100 g	g/100 g	g/100 g
Baklava-1	417 ± 9	50 ± 6	4 ± 0.2	5.1 ± 0.3	5.6 ± 0.4	14.6
Baklava-2	716 ± 8	37 ± 6	7.3 ± 0.1	7.6 ± 0.3	9 ± 0.4	23.9
Baklava-3	427 ± 9	14 ± 1	4.3 ± 0.2	5 ± 0.2	7.2 ± 0.1	16.6
Baklava-4	668 ± 9	211 ± 6	8 ± 0.2	9.1 ± 0.3	10.3 ± 0.2	27.4
Baklava-5	815 ± 6	102 ± 5	7.9 ± 0.2	9.2 ± 0.3	10.3 ± 0.3	27.4
Baklava-6	713 ± 6	358 ± 7	7.6 ± 0.2	9.2 ± 0.1	0.2 ± 0.1	17.0
Baklava-7	552 ± 7	19 ± 1	11 ± 0.2	13.1 ± 0.3	16.6 ± 0.2	40.8
Baklava-8	433 ± 8	50 ± 3	4.5 ± 0.1	4.3 ± 0.2	19.9 ± 0.3	28.8
Baklava-9	722 ± 6	18 ± 2	2.5 ± 0.1	5.1 ± 0.3	16.9 ± 0.5	24.5
Baklava-10	344 ± 8	33 ± 2	2.4 ± 0.1	4 ± 0.3	10.2 ± 0.1	16.5

Values are means ± range, n = 3. GO = glyoxal; MGO = methylglyoxal.

sucrose, and total sugar contents ranged from 2.4 to 11.0 g/100 g, 4.0 to 13.1 g/100 g, 0.2 to 19.9 g/100 g, and 14.6 to 40.8 g/100 g, respectively.

Baklava consists of hard wheat flour, salt, butter, starch, milk, semolina, sugar, eggs, lemon, and nuts. First, the dough is kneaded and then rolled to paper thin thickness with a rolling pin. After that, it is baked in the oven for 40 min at 180 °C and boiled sugar and butter are immediately poured over it (Akkaya & Banu 2017; Savlak & Ergun, 2014). According to the Turkomp (2020), the fat, protein, glucose, fructose, sucrose, and total free sugar content range between 22.5-22.64 g/100 g, 4.10-5.53 g/100 g, 6.44-11.17 g/100 g, 5.50-9.35 g/100 g, 10.14-12.06, and 22.08-32.03 g/100 g, respectively. Compared to Turkomp, the sucrose values of one of the samples was very low (0.2 g/100 g), but with the exception of this one, the lowest sucrose value was 5.5 g/100 g in the remaining samples. As seen from the table, the total sugar content of this sample was 17 g/100 g, and consisting of 99% fructose and glucose. The sugar content of other samples is close to values declared by Turkomp. During caramelization, sucrose can be converted into fructose and glucose with the addition of lemon juice during the heating (Eggleston et al., 1996). However, this conversion can almost be 100% completed with the addition of invertase (Combes & Monsan, 1983). However, according to the Turkish Standard for baklava (Türk Standardlari Enstitüsü, 2015), invertase is not specified at any stage of the baklava formulation (Turkish Baklava Standard). Based on this information and our findings, we thought that this product (baklava 6) may contain high fructose corn syrup (HCFS). HCFS is produced from corn starch via enzymatic reaction and is preferred in the food industry because it is cheaper than sucrose (Parker et al., 2010). Many epidemiologic studies have reported that the consumption of HFCS is associated with increased obesity (Bray et al., 2004; Goran et al., 2013).

As seen from the table, the MGO value of one sample (baklava 6) was very high (358 μg/100 g) compared to other samples. The MGO levels of 7 out of 10 samples were between 14 and 50 μg/100 g, and the value of the other two samples were between 102 and 211 μg/100 g. When we associated the GO and MGO values with the amount of sugar, the MGO value was very high in the samples with low levels of sucrose (0.2 g/100 g). This sample also contained a high level of fructose and glucose. As mentioned above, this sample may be have been formulated with HFCS instead of sucrose. The fructose and glucose levels were still high in the two samples containing a high level of MGO (102 and 211 μg/100 g). With the exception of one sample, it was observed that the MGO values were low in samples with low sugar content. When we evaluated our results, no significant relevance was found between low or high sugar content and GO formation. Unlike the Turkish delight, baklava is made with butter (Akkaya & Banu, 2017), the addition of which may also contribute to GO and MGO formation during processing or prolonged storage.

The most reactive α-DC compounds, GO and MGO, can also result from the caramelization and lipid peroxidation produced by food processing. The MR occurs between reducing sugars and proteins. The presence of protein is required for the MR processes, while it is not required for the caramelization reaction (Uribarri et al., 2010; Sharma et al., 2015).

When we compare the composition of Turkish delight and baklava, the fat and protein content of Turkish delight is very low compared to baklava. For this reason, we thought that the occurrence of GO and MGO in Turkish delight may be caused by caramelization. As seen, in addition to the MR reactions, caramelization, and lipid peroxidation contribute to the occurrence of GO and MGO in baklava.

As mentioned above, the processing condition is 2-5 h at 120 °C for Turkish delight and 40 min at 180 °C for baklava. Caramelization is the common name for a group of reactions that occur when carbohydrates are exposed to high temperatures. Caramelization reaction initiates over 120 °C or pH between 3-9, while the MR starts at temperatures above 50 °C and at pH levels between 4-7 (Kroh, 1994). As seen, the degradation of sugars occurs in two pathways. The sugar concentrations and processing pH are induced by the caramelization. In the first step of caramelization, sucrose hydrolyzes to glucose and fructose (Woo et al., 2015). After that, these products can convert to degradation products such as α-DCs and 5-hydroxymethylfurfural (Khajavi et al., 2005). Therefore, caramelization occurs in both Turkish delight and baklava because both samples contain sugar and have high processing temperatures (over 120 °C). The difference between Turkish delight and baklava is the shelf-life of Turkish delight is about 1 year, while 10 days for baklava (Batu & Kirmaci, 2009; Akkaya & Banu, 2017). As seen, the shelf-life of baklava is very low and the MGO value is also very low compared to Turkish delight (except for one sample). The extent of glycation is accelerated by the increase in processing temperature (Uribarri et al., 2010). As indicated, the MR is initiated between reduced sugar and protein at over 50 °C leading to Amadori products (Kroh, 1994). However, Amadori products can degrade to α-DCs by prolonged storage, cooking time, and increased cooking temperature (Uribarri et al., 2010; Sharma et al., 2015). Increasing the processing temperature from 100 to 120 °C, increases the MGO formation 2 fold (Nedvidek et al., 1992). We thought that since the storage time in baklava is very short, the MGO formation is lower than in that of Turkish delight.

Reduced sugar and unsaturated fatty acids are correlated with increased levels of GO and MGO through lipid oxidation and MR (Jiang et al., 2013). Also, a higher correlation was reported between the level of dicarbonyls and glucose and fructose than sucrose (Amrein et al., 2006). We also thought that GO and MGO were also formed by the MR and lipid peroxidation in baklava because it contains sugar, protein, and lipid.

Lo et al. (2008), reported high levels of GO and MGO in commercial carbonated beverages containing HFCS between 15.8-104.6 and 23.5-139.5 (mg/100 mL), respectively. We thought that one of the baklava samples (sample 6) contained HFCS because the occurrence of MGO was considerably higher than that of the other samples. HFCS is preferred in the food industry due to its high sweetening power, long shelf life, and long-term hydration (Parker et al., 2010). The amount of fructose in HFCS is ranges from 42 to 55% of total sugar and is used as a sweetener in sodas, snacks, beverages, and bakeries (Aragno & Mastrocola, 2017). Some epidemiological studies indicate that there are correlations between the consumption of fructose and insulin resistance, body weight gain, and some other metabolic syndromes (Schulze et al., 2004).

The GO and MGO levels in some foods were 0.2-7 and 0.0-761 mg/kg in honey, 4.8-26.0 and 1.8-81.4 mg/kg in cookies, 0.8-4.0 and 0.2-1.3 mg/kg in oils (cooked) and 0.02-1.73 and 0.07-1.40 mg/kg in carbonated soft drinks, respectively (Wang, 2019). As seen from our results, the GO level is especially high in both Turkish delight and baklava. However, the MGO level is generally high in all Turkish delight and one sample of baklava.

MGO, the precursor of AGEs, is composed of higher reactive dicarbonyl compounds than that of GO. Higher MGO levels cause the inhibition of (IRS)-1 and the PI3K/Akt pathway, resulting in insulin resistance and decreased insulin secretion in beta cells (Matafome et al., 2013). The MGO concentration was found to be very high in the plasma of type 1 and type 2 diabetes individuals In an animal study, the administration of MGO to rats caused beta-cell dysfunction and insulin resistance. The MGO level was found to be between 16-27 μg/dL in diabetic patients while it 3.0-7.0 μg/dL in healthy subjects (Khuhawar et al., 2008). Degen et al. (2012) reported that the estimated range of dietary MGO intake was between 5 and 20 mg/day in people who consumed sugar-rich products. the accumulation of α-DCs can cause cellular damage by reaction with proteins (Nowotny et al., 2015).

These α-DCs lead to the formation of harmful AGEs with the presence of protein (Sharma et al., 2015). The amount of CEL in bread crust, biscuits, and fried dough sticks were reported as 225-820 mg/kg, 15-452 mg/kg, and 146-373 mg/kg, respectively (Wang, 2019). In another study, the amount of CML in bread crust, bread crumb, and biscuits were reported as 58-94 mg/kg protein, 14-34 mg/kg protein, and 50-117 mg/kg protein, respectively (He et al., 2014). Increasing the cooking temperature increases the amount of CML 200 fold in the same food item. The CML level is used to determine the amount of dietary AGE levels in foods. There is a significant correlation between the fat content of foods and MGO with dietary AGE levels (Uribarri et al., 2010).

As seen from our results, both traditional foods (baklava and Turkish delight) contain the precursors of harmful AGEs. The micronutrients such as polyphenols, vitamins A, E, and C, thiamine, and vitamin B_6 are associated with decreased levels of AGEs (Nowotny et al., 2015). One study reported that catechins and proanthocyanidins decrease the level of the MGO, GO, and CML content in fortified bread (Peng et al., 2010). Both baklava and Turkish delight may be formulated with such micronutrients which contain reducing AGEs agents.

4 Conclusions

The AGE precursors GO and MGO, can be formed by Maillard reaction, lipid peroxidation, and caramelization during food processing and prolonged storage. These precursors lead to harmful AGEs as an end result. The increased level of GO and MGO in plasma may cause diabetic complications and Alzheimer's. In this study we observed that, increased sugar concentration, processing time, storage, and HFCS may affect the MGO and GO formations. Since Turkish delight has a considerably longer storage time than baklava, it is thought that the MGO gradually increases over the storage time. Both Turkish delight and baklava contain a high level of sugar and have a higher glycemic index. Therefore, these products should be consumed less or formulated with agents that reduce AGEs.

Conflict of interest

The authors declare that they have no conflict of interest.

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