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Research article

Detection of advanced glycation end product precursors in chocolates enriched with lyophilized cornelian cherry (*Cornus mas* L.)

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Abstract

Advanced glycation end product (AGEs) precursors, glyoxal (GO) and methylglyoxal (MGO), are toxic compounds formed during food processing through the Maillard reaction and, protein and lipid oxidation. Chocolate, a widely consumed product, has been extensively studied for its health effects and contains AGEs and their precursors, which are associated with many chronic inflammatory diseases. Cornelian cherry (*Cornus mas* L.), naturally grown in Türkiye, is rich in antioxidants, vitamin C, anthocyanins, flavonoids, and phenolic compounds. Fruits with natural antioxidant content are known to reduce AGE formation. This study aimed to investigate changes in GO and MGO contents by adding various amounts (10 g, 15 g, and 20 g) of lyophilized *C. mas* powder to different types of chocolate (dark, milk, and white). AGE precursors analysis was performed using High-Performance Liquid Chromatography (HPLC). Additionally, sensory analysis was conducted to determine the consumption potential of the chocolates. Fourteen panelists aged 18-65 evaluated the chocolate samples using a single-blind method by tasting the samples and completing a sensory analysis questionnaire. Data were evaluated and reported using the SPSS 26.0 software package. GO contents of the samples ranged from 14.0 to 268.6 µg/100g, while MGO contents ranged from 122.3 to 284.0 µg/100g. It was observed that only in milk chocolate samples did the GO content decrease with increased amounts of *C. mas*. In the sensory analysis, among chocolate groups, the most preferred product after the control groups was white chocolate with 10 g (3.86 ± 0.86). Significant differences were found among chocolate types in terms of taste, bitterness, melting in the mouth, texture, hardness, sourness, and overall acceptance (p<0.05). Foods with high antioxidant content, such as *C. mas* L. affect the AGE precursors in products. More comprehensive studies examining the antioxidant capacity concerning GO and MGO determination in chocolate types are needed.

Keywords: *Advanced glycation end products; chocolate; cornelian cherry; Cornus mas; glyoxal; methylglyoxal*

1. Introduction

Advanced glycation end products (AGEs) are formed through a non-enzymatic reaction between reducing sugars and free amino groups of proteins, lipids, or nucleic acids (Uribarri et al., 2010). This non-enzymatic reaction is commonly known as Maillard or browning reaction (O'Brien and Morrissey, 1989; Uribarri et al., 2010; Zhu et al., 2024). The main AGE precursors formed in the process of Maillard reactions are glyoxal (GO), methylglyoxal (MGO), and 3-deoxyglucosone (Sharma et al., 2015; Nowotny et al., 2018). These precursors are called α-dicarbonyl compounds, and these compounds interact with the

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amino groups of proteins to form AGEs (Cengiz et al., 2020).

The formation of AGEs is a natural part of human metabolism, but the excessive contents of AGEs in tissues and circulation can lead to pathogenic effects in the body (Ulrich, 2001; Uribarri, 2010). In addition to the formation of AGEs within the human body, these compounds are also ingested through the consumption of food. Clinical studies show that dietary AGEs may increase risk factors associated with chronic diseases such as inflammation, oxidative stress, insulin resistance, diabetes, kidney diseases, and cardiovascular and cerebrovascular disorders (Goldberg et al., 2004; Uribarri et al., 2010; Wei et al., 2018; Tian et al., 2023; Zgutka et al., 2023). Almost all foods, such as chocolate, sugary products, bread, coffee, baby food, and breast milk, contain glycation products to a lesser or greater extent (Singh et al., 2001; Zhang et al., 2024).

According to the literature, it is seen that the determination of AGE precursors in major processed products such as chips, crackers, and packaged cakes has been carried out (Cengiz et al., 2020; Catak and Balci, 2022). Conversely, no study was identified in the existing literature that has determined and compared AGE precursors in different types of chocolate, which are consumed by individuals across a wide age range.

Chocolate is a widely consumed flavorful product and is the subject of many researchers due to its positive or negative effects on health. There is evidence suggesting that cocoa, the primary component of chocolate, has positive effects on the heart, is beneficial for the liver, aids digestion, and improves sleep (Dilinger et al., 2000; Comert and Merdol., 2018). Today, scientific studies on the effects of cocoa and chocolate on risk factors associated with Type 2 diabetes mellitus, cardiovascular diseases, blood lipid profile, antioxidant capacity, insulin resistance, blood pressure, inflammation, and obesity have become widespread (Bruinsma and Taren, 1999; Katz et al., 2011; Ellam and Williamson, 2013; Comert and Merdol, 2018). The adverse health effects of commercially available chocolates are generally analyzed in terms of the amount of sugar and additives they contain. It is known that these products contain AGEs and AGE precursors, which are known to have a negative effect on many chronic inflammatory diseases (Javed et al., 2021; Yan et al., 2023).

Two strategies have been proposed to reduce α-dicarbonyl compounds, which are one of the AGE precursors in foods. The first one is to use non-thermal technologies in food processing, and the second is to optimize the formulation of food components by adding phenolic compounds. However, it should be noted that these approaches may affect the organoleptic properties and nutritional value of foods (Javed et al., 2021; Yan et al., 2023; Kou et al., 2024).

There are studies supporting the hypothesis that red-purple fruits, known for their antioxidant properties, inhibit AGE formation (Chen et al., 2014; Yusufoglu et al., 2020; Hsiao et., 2024; Tan et al., 2024). Studies on Cornelian cherry (*C. mas* L.; CM) fruit, which is one of these red fruits, and its health benefits have increased in recent years (Celik et al., 2023; Bayram et al., 2024a; Bayram et al., 2024b; Szot et al., 2024). CM is thought to contribute to a healthy diet due to its phenolic components, organic acids, vitamin C, pectins, carotenoids, and essential minerals. Antioxidant, antimicrobial, antidiabetic, anti-atherosclerosis, anti-obesity, anti-glaucoma, cytoprotective, neuroprotective, cardioprotective, liver and kidney protective properties, hypolipidemic and hypotensive effects of CM have been noted (Bayram and Ozturkcan, 2020; Lidiková et al., 2024).

In the literature, studies on products enriched with CM mostly refer to the antioxidant content of the products and do not include the effects on the content of AGE precursors. For this reason, this study aimed to investigate the formation of AGE precursors in different types of chocolate (white, milk, dark) by adding lyophilized dried CM. Moreover, the organoleptic properties of the chocolates were also evaluated by sensory analysis (Lidiková et al., 2024; Bayram et al., 2024a).

2. Materials and methods

2.1. Preparation of samples

The CM fruits were collected from the Uzundere district of Erzurum province, pureed, and lyophilized by sublimation method in Ray 125 Freeze Dry machine with 300 tray capacities. Milk, dark (54.0% cocoa content), and white couverture chocolates of a brand that is widely used as chocolate and available on the market shelves were purchased. Declared macronutrient values of the chocolate samples are shown in Table 1.

Table 1
Declared macronutrient values of the chocolate samples.

Sample	Carbohydrate (g/100 g)	Protein (g/100 g)	Fat (g/100 g)
Milk Chocolate	61	6	27
Dark Chocolate	48	6.1	34
White Chocolate	58	3.9	36

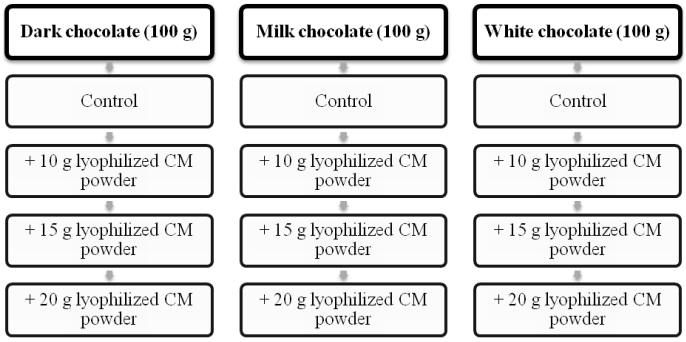

Fig. 1. Preparation of chocolate samples.

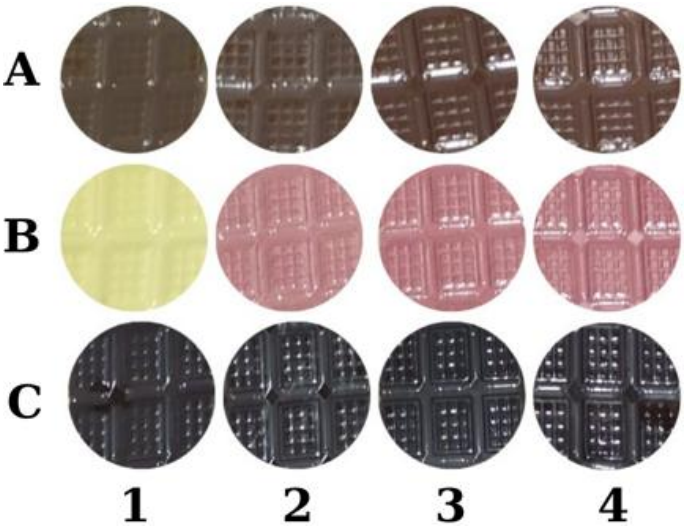

Fig. 2. Chocolate samples. (A) milk chocolate, (B) white chocolate, (C) dark chocolate, (1) control, (2) 10 g CM added, (3) 15 g CM added, (4) 20 g CM added.

All samples, with or without the addition of CM, were processed in the same way, melted in a bain-marie and then poured into chocolate molds and frozen. Determined amounts of CM were added to each chocolate flavor separately.

The chocolates were melted in 100 g samples in a bain-marie. Dark chocolate melted at 63°C, milk chocolate at 51°C, and white chocolate at 53.6°C. To preserve the vitamin C content of CM, the temperature of the melted chocolates was not high. Temperature control was done with a probe thermometer. Lyophilized CM powder in different amounts was added to the chocolates at 40°C and mixed (Fig. 1). The mixed chocolates were poured into silicone chocolate cups and placed in the freezer. The frozen chocolates were vacuumed and placed in cooler bags for analysis (Fig. 2).

2.2. Chemical analyses of AGE precursors in samples

AGE precursors of 12 different samples were analyzed by the HPLC method using 4-nitro-1,2-phenylenediamine as a pre-column derivatization reagent, a method used in the studies of Cengiz et al. (2020) and Catak and Balci (2022). GO and MGO contents were analyzed as AGE precursors in the samples.

2.2.1. Extraction and derivatization of GO and MGO

The extraction of GO and MGO in the samples was carried out according to the method described by Cengiz et al. (2020) with some modifications. All samples were homogenized and then 5 g of each sample was taken and placed in a 50 ml falcon tube. 5 ml of methanol was added. The samples were extracted for 2 min using an Ultra Turrax homogenizer (IKA, Staufen, Germany) and centrifuged at 8000 rpm for 5 min. After centrifugation, 0.5 ml of liquid samples were taken into 10 ml glass tubes, and 1 ml of sodium acetate buffer (0.1 M, pH: 3) was added to the samples. After adding 0.5 ml of derivatization solution (4-nitro-1,2-phenylenediamine in 1% methanol) to the samples, the resulting mixtures were incubated at 70°C for 20 min and filtered through cellulose acetate filter (pore size 0.45 µm) and injected into HPLC.

2.2.2. HPLC determination of GO and MGO

The HPLC conditions described by Cengiz et al. (2020) were modified. The HPLC system consisted of a Shimadzu SPD-20A UV/VIS detector (Shimadzu Corporation, Kyoto, Japan), and a Shimadzu LC 20AT pump was used. The mobile phase was methanol:water: acetonitrile (42:56:2, v/v/v). The wavelength was 255 nm. GO and MGO were separated by 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 set to 30°C.

2.3. Sensory Analysis of the Samples

For sensory analysis, the samples were evaluated by trained panelists using the single-blind method. During the sensory analysis, the sample names were not openly written, the samples were assigned random codes, and the panelists were presented with the samples in a random order. The panelists were blinded to the samples they tasted. The panelists were between the ages of 18-65 and included 14 participants. Analyses were performed according to the matched-comparison method. A questionnaire containing, color, smell, flavor, bitter taste, oily taste, texture, hardness, stickiness, melt in the mouth, sourness, and general acceptability criteria of the products were scored by 5-point Likert method (very good, good, neutral, bad and very bad). Panelists were asked to neutralize the aftertaste with water before tasting each sample. An informed consent form was signed by all participants before starting the sensory analysis. The sensory analysis of this study was approved ethically by the Marmara University Faculty of Health Sciences Non-Invasive Clinical Studies Ethics Committee (Protocol no: 2022/181), and the research was conducted following the principles stated in the Helsinki Declaration.

2.4. Statistical Analyses

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

3. Results

3.1. MGO and GO analyses of samples

As shown in Table 2, the MGO content of the samples varied between 122.3-284.0 µg/100 g. There is a significant difference between chocolate types and MGO content ($p<0.05$). The sample with the highest MGO content was white chocolate 10 g (284.0 µg/100 g) and the sample with the lowest MGO content was milk chocolate 10 g (122.3 µg/100 g). White chocolate was found to have the highest MGO content among the control samples (240.6±11.0 µg/100 g). Following white chocolate, the order was milk chocolate and dark chocolate (196.6±9.0 µg/100 g and 140.3±6.5 µg/100 g, respectively). Among the dark chocolate samples, the highest MGO content was found in the sample with 20 g CM addition (271.0 µg/100 g), while the lowest contents were found in the samples with 10 g CM addition (140.3 µg/100 g) and control (140.3 µg/100 g). The analysis revealed that among the milk chocolate samples, the sample with 10 g CM addition (122.3 µg/100 g) contained statistically significantly lower MGO than the other milk chocolate samples. Of the white chocolates, the MGO content was found to be statistically significantly highest in the white chocolate with 10 g CM addition (284.0 µg/100 g).

Table 2
MGO content of samples.

Chocolate Sample	Mean±SD (µg/100 g)	95% CI	*p* Value
Dark chocolate - control	$140.3^{a,b}$±6.5	124.1-156.4	< 0.001
Dark chocolate + 10 g CM	$140.3^{a,b}$±6.5	124.1-156.4	
Dark chocolate + 15 g CM	$167.3^{b,c}$±7.5	148.6-185.9	
Dark chocolate + 20 g CM	$271.0^{f,g}$±12.5	239.8-302.1	
Milk chocolate - control	196.6^{d}±9.0	174.2-219.0	
Milk chocolate + 10 g CM	122.3^{a}±5.5	108.6-136.0	
Milk chocolate + 15 g CM	$193.6^{c,d}$±9.0	171.2-216.0	
Milk chocolate + 20 g CM	211.0^{d}±9.5	187.3-234.6	
White chocolate - control	240.6^{e}±11.0	213.3-268.0	
White chocolate + 10 g CM	284.0^{g}±12.5	252.8-315.1	
White chocolate + 15 g CM	$251.0^{e,f}$±11.5	222.3-279.6	
White chocolate + 20 g CM	241.6^{e}±11.0	214.3-269.0	

CM: *Cornus mas*. L.; SD: Standard Deviation; CI: Confidence Interval. The one-way analysis of variance (ANOVA) was used. There is a significant difference between values with different letters in the superscripts in the column.

Table 3
GO content of samples.

Chocolate Sample	Mean±SD (µg/100 g)	95% CI	*p* Value
Dark chocolate - control	268.6^{h}±12.0	238.8-156.4	< 0.001
Dark chocolate + 10 g CM	51.3^{c,d}±2.5	45.0-156.4	
Dark chocolate + 15 g CM	63.0^{d,e}±3.0	55.5-185.9	
Dark chocolate + 20 g CM	61.3^{c,d,e}±2.5	55.0-302.1	
Milk chocolate - control	113.3^{g}±5.5	99.6-219.0	
Milk chocolate + 10 g CM	67.0^{e}±3.0	59.5-136.0	
Milk chocolate + 15 g CM	63.0^{d,e}±3.0	55.5-216.0	
Milk chocolate + 20 g CM	37.3^{b}±1.5	33.3-234.6	
White chocolate - control	14.0^{a}±1.0	11.5-268.0	
White chocolate + 10 g CM	48.0^{b,c}±2.0	43.0-315.1	
White chocolate + 15 g CM	86.3^{f}±3.5	77.6-279.6	
White chocolate + 20 g CM	85.3^{f}±3.5	76.6-269.0	

CM: *Cornus mas*. L.; SD: Standard Deviation; CI: Confidence Interval. The one-way analysis of variance (ANOVA) was used. There is a significant difference between values with different letters in the superscripts in the column.

The GO content of the samples ranged from 14.0-268.6 µg/100g (Table 3). There was a significant difference between chocolate types and GO contents (p<0.05). Among the control groups, dark chocolate had the highest GO content (268.6±12.0 µg/100 g). Milk chocolate and white chocolate followed by dark chocolate (113.3±5.5 µg/100 g and 14.0±1.0 µg/100 g, respectively). Among the dark chocolate samples, the highest GO content was found in the dark control (268.6 µg/ 100 g) and the GO content of all dark samples to which CM was added was statistically lower than that of the control sample. Among the milk chocolate samples, the highest amount of GO was found in the control milk chocolate (113.3 µg/100 g), and the lowest amount was found in the milk chocolate with 20 g CM (37.3 µg/100 g), and there was a statistically significant difference between them. Among the white chocolate samples, the highest GO content was found in the samples with 15 g CM (86.3 µg/100 g) and 20 g CM (85.3 µg/100 g), while the lowest GO content was found in the control chocolate (14.0 µg/100 g).

3.2. Sensory analysis results of samples

For sensory analysis, the samples were evaluated by trained panelists using the single-blind method. The panelists were between the ages of 18-65 and included 14 participants. The results of sensory analysis of dark, milk and white chocolate samples were presented as mean scores in Table 4. According to the general acceptance criteria, it was determined that the most liked sample after the control groups was 10 g CM added white chocolate (3.86±0.86). Taste, bitter taste, melt-in-the-mouth, texture, hardness, sour taste, and general acceptance criteria showed differences between the samples (p<0.05).

Among the dark chocolate samples, the most liked sample in terms of overall acceptance was the control sample (4.29±0.83), while the least liked sample was the 20 g CM added dark chocolate (3.07±1.07). In terms of the bitter taste criterion, the most bitter sample was the control dark chocolate (4.21±0.89). Among the dark chocolates, the dark chocolates with 10 g CM and 20 g CM (3.36±0.93 and 3.36±1.22, respectively) scored the lowest in terms of texture. When they were evaluated in terms of sourness, the control sample (4.36±1.08) received the highest appreciation, and the samples with 15 g CM added, and 20 g CM added (2.79±0.89 and 2.86±1.10, respectively) received the lowest score.

Table 4
Sensory analysis results of chocolate samples.

Criteria	Samples												*p*
	Dark chocolate				Milk chocolate				White chocolate				
	C	10 g	15 g	20 g	C	10 g	15 g	20 g	C	10 g	15 g	20 g	
Color	4.57±0.51	4.29±0.73	4.64±0.63	4.36±0.84	4.71±0.47	4.29±0.83	3.93±0.83	3.50±1.40	4.29±0.83	4.36±0.93	4.43±0.76	4.50±0.65	0.091
Smell	4.50±0.52	4.00±0.68	4.29±0.61	3.93±0.83	4.50±0.52	4.29±0.47	4.07±0.73	3.93±1.00	4.14±0.66	4.14±0.86	3.86±0.86	3.86±1.10	0.395
Taste	4.43^{b}±1.09	3.29^{a}±0.99	3.50^{a,c}±1.09	3.14^{a}±1.17	4.29^{b,c}±0.83	3.57^{a,c}±0.76	3.07^{a}±1.00	2.93^{a}±1.07	3.57^{a,c}±1.22	3.71^{a,b,c}±1.14	3.14^{a}±1.17	3.29^{a}±1.14	0.002
Bitter taste	4.21^{c}±0.89	3.29^{a}±1.38	3.50^{a,b}±1.22	3.43^{a,b}±1.09	4.36^{c}±0.84	3.43^{a}±0.65	3.21^{a}±0.97	3.21^{a}±1.12	4.07^{b,c}±0.62	4.21^{b,c}±0.70	3.29^{a}±1.07	3.50^{a,b}±0.94	0.002
Oily taste	4.14±1.03	3.64±1.15	3.50±1.09	3.57±1.02	3.64±1.15	3.57±0.85	3.07±1.00	2.93±1.33	3.21±1.31	3.43±1.09	3.29±1.20	3.50±1.09	0.316
Melting in the mouth	4.36^{b}±0.84	3.43^{a}±1.02	3.50^{a}±1.02	3.36^{a}±1.15	4.21^{b}±0.89	3.21^{a}±1.12	3.00^{a}±1.04	2.86^{a}±1.10	4.29^{b}±0.61	3.43^{a}±1.02	3.21^{a}±1.05	3.00^{a}±1.11	<0.001
Texture	4.43^{b}±0.85	3.36^{a}±0.93	3.64^{a}±1.08	3.36^{a}±1.22	4.50^{b}±0.65	3.36^{a}±0.93	3.14^{a}±0.95	3.07^{a}±0.92	4.43^{b}±0.65	3.71^{a}±0.91	3.14^{a}±1.03	3.14^{a}±1.17	<0.001
Hardness	4.50^{b}±0.65	3.79^{a}±0.89	3.79^{a}±1.19	3.86^{a}±1.03	4.14^{b}±0.86	3.93^{a}±0.83	3.36^{a}±1.01	3.14^{a}±1.03	4.29^{b}±0.83	4.14^{b}±0.77	3.64^{a}±0.93	3.36^{a}±0.84	<0.001
Stickiness	4.00±1.18	3.71±1.20	3.43±0.94	3.50±1.02	3.86±1.03	3.79±0.70	3.36±0.93	3.21±1.05	4.07±0.83	3.86±0.77	3.57±0.85	3.43±0.76	0.188
Sour taste	4.36^{b}±1.08	2.93^{a,c}±1.27	2.79^{a}±0.89	2.86^{a,c}±1.10	4.21^{b}±0.89	2.93^{a,c}±1.07	2.93^{a,c}±0.92	2.43^{a}±0.85	4.07^{b}±0.83	3.64^{b,c}±0.93	2.93^{a,c}±1.14	3.21^{a,c}±0.97	<0.001
Overall Acceptance	4.29^{b}±0.83	3.36^{a,c}±1.08	3.50^{a,c,d}±1.02	3.07^{a}±1.07	4.29^{b}±0.61	3.64^{a,b,c,d}±0.74	3.29^{a,c}±0.91	2.93^{a}±1.07	4.07^{b,d}±1.00	3.86^{b,c}±0.86	3.21^{a,c}±0.80	2.93^{a}±0.92	<0.001

C; control. Values are expressed as mean ± standard deviation. The one-way analysis of variance (ANOVA) was used. There is a significant difference between values with different letters in the superscripts in the rows (p < 0.05).

In terms of overall acceptance of the milk chocolate samples, the control chocolate had the highest score (4.29±0.61), while the milk chocolate with 20 g CM added had the lowest score (2.93±1.07). When the milk chocolate samples were evaluated in terms of taste and fatty taste criteria, it was found that the sample with 20 g CM added received the lowest score (2.93±1.07 and 2.93±1.33, respectively). The milk chocolate sample with the best melt-in-the-mouth score was the control chocolate (4.21±0.89). Among these chocolates, in terms of texture criterion, the milk chocolate with 20 g CM added had the lowest score (3.07±0.92). For sourness, the highest score was given to the control sample (4.21±0.89), and the lowest score was given to the sample with 20 g CM (2.43±0.85).

In terms of overall acceptance of the white chocolate samples, the control sample received the highest score, while the sample with 20 g CM added received the lowest score (4.07±1.00 and 2.93±0.92, respectively). Among the white chocolates, the sample that had the lowest score in terms of taste was the sample to which 15 g CM was added (3.14±1.17). According to the melting in the mouth and texture criteria, the white chocolate sample with the highest score was the control (4.29±0.61 and 4.43±0.65, respectively), while the sample with the lowest score was the sample with 20 g CM (3.00±1.11 and 3.14±1.17, respectively). In terms of sourness, it was determined that the sample with the highest score was the control (4.07±0.83) and the lowest score was the white chocolate with 15 g CM (2.93±1.14).

4. Discussion

This study investigated the GO and MGO contents and sensory parameters of dark, milk, and white chocolates to which different amounts of lyophilized cornelian cherry (*C. mas* L.) powder were added. Among the tested samples, milk chocolate showed a notable reduction in GO levels as the number of *C. mas* L. powder increased, highlighting its potential role in mitigating AGE formation. Sensory analysis revealed that while control samples were most favored, white chocolate with 10 g *C. mas* L. powder was the most acceptable among the enriched samples.

The main ingredients of chocolate are cocoa butter, cocoa powder, milk, and sugar. The formation of AGEs in chocolate is dependent on several factors, including lipid oxidation, sugar autoxidation, nutrient composition, moisture content, pH, and thermal processing conditions. Prolonged storage can also influence the formation of AGEs. Cintesun et al. (2022) found that the content of GO and MGO in chocolates varied between 20-258 µg/100 g and 50-274 µg/100 g, respectively. The contents of GO and MGO in the chocolates used in our study were found to vary between 14-268.6 µg/100 g and 122.3-284 µg/100 g, respectively. It is thought that these differences in the results are due to the type, content, and brands of chocolates used.

It has been reported that the MGO and GO contents of foods with high carbohydrate content are high. In addition to carbohydrates, it has been shown in the literature that there is a positive correlation between the fat and protein contents of foods and MGO and GO contents (Yilmaz and Karabudak, 2016; Maasen et al., 2021). Among the chocolates used in this study, although milk chocolate had the highest carbohydrate content, MGO and GO contents of milk chocolate were lower than dark and white chocolate. It is thought that this may be due to the fact that milk chocolate has the lowest fat content. It was observed that MGO contents were generally high in white chocolate samples, which had the highest fat content among the chocolate types used in this study. In addition, an increase in GO contents was detected in proportion to the amount of CM added. Since white chocolate contains cocoa butter rather than cocoa beans, it is thought to have very low polyphenols and therefore low antioxidant capacity. For this reason, CM-added chocolates prepared with white chocolate may have been insufficient to inhibit AGE precursors formed during production.

The reducing effect of polyphenols on AGE formation is based on increasing the antioxidant capacity of the added food and binding dicarbonyls (Yalcin and Rakicioglu, 2022). In a study by Ede-Cintesun et al. (2024), the GO and MGO contents of different types of chocolate available in the markets were analyzed and it was shown that the GO content of white chocolate containing raspberries was lower and the MGO content was higher than plain white chocolate. In the same study, it was reported that dark chocolate containing mulberry had higher GO and MGO content than plain dark chocolate (Ede-Cintesun et al., 2024). In a study conducted by Yusufoglu et al. (2020), it was reported that fruit-based heat-treated foods contain high amounts of AGE precursors and the reason for this was caramelization and sugar autooxidation occurring during production (Yusufoglu et al., 2020). In the study of Karatay (2022), it was observed that the highest GO content among the protein bars in the market was in the forest fruit protein bar and it was thought that the reason for this may be the fructose contents in the heat-treated fruit. Cintesun et al. (2022) also found that fruit cake had the highest GO and MGO contents among cakes in a study on snacks. In our study, the drying of CM by lyophilization method and the fact that the chocolates were below 40°C when added to the chocolates ensured that the heat treatment of CM was kept at a minimum level. It is thought that the decrease in GO contents in milk and dark chocolate samples to which CM was added may be related to this. However, despite antioxidant components like polyphenols in cocoa and CM, it was observed that MGO contents in the samples did not decrease and even showed an increase. These differences in the results may be due to the complex composition of the chocolates and the increased carbohydrate content with the added CM.

Although CM added to different chocolate samples has been shown to affect MGO and GO contents, it is also important to evaluate the consumability of these chocolates. In a study conducted by Uzumcu and Ozsisli (2023), varying quantities of blueberries and oatmeal were incorporated into milk chocolates. The results of the sensory analysis indicated that the control groups received the highest scores, while the chocolates containing 40% and 50% additives received the lowest scores. Furthermore, the impact of oatmeal and blueberry-added chocolates on overall acceptability was found to be statistically insignificant. In another study, pomegranate seeds and pomegranate jelly were added to white and milk chocolates to investigate the sensory properties of these chocolates, and the white chocolate sample with pomegranate jelly received the highest scores in terms of color, smell, and taste. However, in terms of consumption and consumer acceptability, it was noted that milk chocolate with pomegranate jelly was the most preferred chocolate sample overall (Yildirim et al., 2016). According to our results of the sensory analyses, it was found that milk chocolate with 10 g CM, which was one of the most successful samples in terms of reducing MGO and GO contents, was also similar to the milk control in terms of overall

acceptability. Among the chocolates to which CM was added, 10 g CM added milk chocolate had the highest score after 10 g CM added white chocolate. The reason for the 10 g CM added white chocolate having the highest overall score is that it has the highest score in terms of color. On the other hand, the sample with the lowest appreciation score was found to be dark chocolate with 20 g CM powder added. Nevertheless, in contrast to the findings of the study by Uzumcu and Ozsisli (2023), the impact of CM-added chocolate groups on overall acceptability was determined to be statistically significant. However, similar to the study by Yildirim et al. (2016), the addition of a red fruit increased the acceptability of white chocolates in terms of color criterion.

The inclusion of three different control groups in this study enabled comparisons with the test groups and increased the reliability of the data obtained. However, the analysis of AGE precursors (MGO and GO) instead of AGEs presents a limitation, as it does not directly quantify the content of AGEs in the products. Moreover, it does not allow for determining the extent to which these precursors are converted into AGEs within the organism. Another limitation is that only one brand of chocolate was analyzed, which restricts the generalizability of the findings. Future research could address this limitation by including chocolates from different brands and with varying cocoa ratios. It is also recommended that future studies analyze the total antioxidant capacity and anthocyanin content of chocolates to obtain more accurate and comprehensive results. Nonetheless, to the best of our knowledge, no previous studies have focused on determining AGE precursors in cornelian cherry (*C. mas* L.) powder-enriched chocolate samples. This study represents a novel approach, combining the determination of AGE precursors and sensory analysis for chocolates enriched with *C. mas* L. powder, providing a foundation for more extensive research in this area.

5. Conclusion

As a result of the analysis of the samples prepared in the study, it was observed that the MGO amounts were generally high, but the GO content decreased. It is known that foods with high antioxidant content, such as CM, affect the AGE content. For this reason, new studies are necessary to better understand the reasons for these changes in MGO and GO. When we look at the literature and the results of this study, one area requiring more detailed examination is the formation mechanism of MGO and GO. Upon examining the contents of these two AGE precursors, which share similar pathways, in chocolate samples, it is observed that there is no consistent decrease, particularly in dark and white chocolates, with MGO contents remaining elevated. However, assessing the total antioxidant capacity of chocolate and cornelian cherry may offer a more accurate interpretation on this subject.

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Ethical Approval: Sensory analysis of this study was approved ethically by the Marmara University Faculty of Health Sciences Non-Invasive Clinical Studies Ethics Committee (Protocol no: 2022/181) and the research was conducted following the principles stated in the Helsinki Declaration.

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