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From bite to bioaccessibility: in vitro bioaccessibility of malondialdehyde and hydroxymethylfurfural from cookies

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Summary

This study aimed to investigate the formation of malondialdehyde (MDA) and hydroxymethylfurfural (HMF) in cookies under in vitro digestive system conditions. For that purpose, MDA and HMF levels were analysed using high-performance liquid chromatography (HPLC) at initial and after in vitro digestion. Initial MDA and HMF values were found to range between 0.17 ± 0.01 mg·kg^{-1} to 1.01 ± 0.03 mg·kg^{-1} and 0.9 mg·kg^{-1} to 175.1 mg·kg^{-1}, respectively. The highest MDA and HMF levels at initial were found in tahini and white chocolate-containing samples. After digestion, the bioaccessibility of MDA and HMF ranged from 17 ± 1 % to 329 ± 10 % and from 0 ± 0 % to 200 ± 8 %, respectively. Decreased bioaccessibility of MDA and HMF was observed in most of the cookies. However, in gluten-free cookies, both MDA and HMF were detected at the highest levels of bioaccessibility. Further studies should focus on MDA and HMF formation under digestive system conditions, including the colon, to determine the effects of gut microbiota.

Keywords

cookies; malondialdehyde; hydroxymethylfurfural; in vitro; bioaccessibility

Nowadays, with the changes in dietary habits, the consumption of processed food has increased, especially as bakery products are an essential part of this consumption. Among bakery products, cookies are both nutritious and appealing to a wide range of consumers [1]. Cookies contain flour, sugar, oil, eggs, and salt as the main ingredients and are processed at temperatures up to 220 °C during the production process, which may enable the production of thermal process contaminants [2]. High sugar and fat content, low moisture, pH, processing conditions at high temperatures, and prolonged shelf life facilitate the formation of 5-hydroxymethylfurfural (HMF) and malondialdehyde (MDA) in cookies [3].

MDA and HMF are reactive compounds found in heat-treated foods and can have significant health effects. HMF is a reactive compound formed when sugars are exposed to high heat treatment or acidic conditions. It is usually formed during the Maillard reaction and caramelisation processes, especially when sugars such as fructose and glucose lose water in high-temperature and acidic environments and transform into a five-membered furfural ring [4]. MDA is a lipid peroxidation by-product formed by the oxidation of polyunsaturated fatty acids (PUFAs) reacting with free radicals and can have mutagenic and carcinogenic effects that can lead to genetic damage. Therefore, determining the amount of these compounds in foods is critical for food safety and public health [5].

MDA and HMF can be easily formed in biscuits due high-temperature cooking conditions,

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sugar and fat content [2, 6]. High levels of MDA and HMF can increase the risk of cardiovascular diseases, cancer, liver and kidney diseases, neurodegenerative disorders and metabolic diseases through increased oxidative stress, DNA damage and impaired cellular function [3, 5, 7]. Moreover, digestive tract conditions may affect the formation of MDA and HMF in foods, depending on factors including pH, temperature, oxygen, metallic ions, enzymatic activities and chemical reactions [8]. The effect of the digestive system on the formation of HMF and MDA is controversial in the literature. Existing studies have examined the effects of the digestive process on the formation and bioaccessibility of these compounds in diet bars, meat, cake, coffee or bread [9–14]. To our knowledge, there is a lack of research focused on the formation of HMF and MDA in biscuits. Therefore, the present study aims to investigate the formation of HMF and MDA in biscuits under in vitro digestive system conditions.

MATERIALS AND METHODS

Samples

A total of 22 different cookie samples were purchased from commercial markets in Istanbul, Türkiye. The energy and nutrient values, including fat, carbohydrate, sugar, protein, salt, and fibre declared on the label, were presented in Tab. 1.

Chemicals

Trichloroacetic acid (TCA), thiobarbituric acid (TBA), tetraethoxypropane, 5-hydroxymethylfurfural (HMF), ethanol, methanol, pancreatin (8× USP specifications from pig pancreas), lipase (Type II from pig pancreas, 1.0–5.0 × 10^8 U·kg^{-1} protein), alpha-amylase (from *Aspergillus oryzae* powder, 1.5 × 10^6 U·kg^{-1}), pepsin (from pig gastric mucosa solid, lyophilised powder, 2.5 × 10^8 U·kg^{-1}), mucin, bile salts, and distilled water were utilised as reagents in the experimental procedures. All chemicals were of analytical grade and obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

Analysis of malondialdehyde

MDA analysis was performed by high-performance liquid chromatography (HPLC) after derivatisation with TBA. Briefly, 2 g of sample was weighed into a 50 ml Falcon tube. Then, 25 ml of 10% TCA was added and vortexed for 1 min. The mixture was centrifuged at 10 000 ×g for 10 min. After centrifugation, 1 ml of the supernatant and 1 ml of 6.7 g·l^{-1} TBA solution were mixed. The mixture was vortexed and incubated in a water bath at 95 °C for 30 min to form the MDA-TBA complex. It was then cooled to room temperature and centrifuged at 5 000 ×g for 10 min. The resulting supernatant was filtered through a 0.45 μm membrane filter and injected into the Shimadzu Nexera-i HPLC system equipped with a Shimadzu RF-20A fluorescence detector (Shimadzu, Kyoto, Japan). Separation was performed using a Gemini-NX C18 column (5 μm, 11 nm, 4.6 × 250 mm; Phenomenex, Torrance, California, USA) with a mobile phase consisting of a mixture of 0.05 mol·l^{-1} KH_2PO_4 buffer solution, methanol and acetonitrile (72 : 17 : 11, v/v/v). Detection of the MDA-TBA complex was performed with a Shimadzu Nexera-i HPLC with a fluorescence detector, with excitation at 530 nm and emission at 550 nm. The external standard calibration curve, generated with tetraethoxypropane (TEP), the precursor standard of MDA, was used for MDA quantification.

Analysis of hydroxymethylfurfural

HMF levels were determined by HPLC. Prior to analysis, 1 g of digested cookie sample was homogenised with 10 ml of methanol and centrifuged at 5 000 ×g for 10 min. The resulting supernatant was passed through a 0.45 μm membrane filter and injected into the Shimadzu HPLC 20 AT pump with a Shimadzu SPD-20 A UV/VIS detector (Shimadzu). Analyses were carried out using a Gemini-NX C18 column (5 μm, 11 nm, 4.6 × 250 mm; Phenomenex); a mixture of water and methanol (typically 92 : 8, v/v) was applied as the mobile phase in isocratic mode with a flow rate of 1.0 ml·min^{-1}. HMF was detected with a UV detector at a wavelength of 280 nm. The amount of HMF was calculated from an external standard calibration curve using a pure HMF standard from Sigma-Aldrich.

Gastrointestinal process and bioaccessibility

The gastrointestinal processes and bioaccessibility of MDA and HMF were evaluated according to the method proposed by Brodkorb et al. [15]. The composition of digestive juices, origin of enzymes, and their activities were the same as described in the harmonised protocol by Minekus et al. [16]. First, 5 g of sample was weighed into a 50 ml Falcon tube and sequentially exposed to the oral phase for 5 min, the gastric phase for 2 h, and the intestinal phase for 2 h at 37 °C. After that, the digested mixture was centrifuged at 10 000 ×g for 10 min. The amounts of MDA and HMF were analysed in the digested solution, and their bioaccessibility was calculated.

Tab. 1. The macro and micronutrient contents of the cookie samples.

No	Cookie	Energy		Fat [g·kg^{-1}]	Saturated fat [g·kg^{-1}]	Carbohydrate [g·kg^{-1}]	Sugar [g·kg^{-1}]	Protein [g·kg^{-1}]	Salt [g·kg^{-1}]	Fibre [g·kg^{-1}]
		[kcal·kg^{-1}]	[kJ·kg^{-1}]							
1	Coconut, chocolate chips	5210	21793	278	139	611	241	66	1	nd
2	Gum drops, powdered sugar	4780	19999	210	120	640	200	72	9.2	20
3	Butter	4920	20585	230	110	630	210	76	8.1	19
4	Butter	5760	24099	279	144	744	277	77	6	16
5	High fibre, raspberry, apple, hazelnut, no added sugar	4670	19539	244	142	518	102	79	6	143
6	Chocolate chips	4470	18702	216.9	82.7	558.8	152.5	59,1	2.3	21.9
7	Lemon, white chocolate	5000	20920	250	210	620	220	66	6.8	9
8	Tahini, crispy	5660	23681	401	54	409	175	146	1.86	nd
9	Gum drops	5260	22007	273	66	623	201	83	0.09	nd
10	Kavala	5330	22300	265	62	603	228	90	1	nd
11	Chocolate	4720	19748	240	110	580	300	57	4.8	nd
12	Blueberry, cranberry	4570	19120	200	88	630	290	50	5.3	nd
13	White chocolate, cranberry	4890	20459	230	110	640	450	51	6.7	nd
14	Tahini	5520	23095	336.4	84.6	538	262.2	98	1	nd
15	Tahini, nut	5520	23095	336.4	98	538	262.2	84.6	1.3	nd
16	Nut, bitter almond	4890	20459	238	18	609	544	77	2	nd
17	Sunflower seed, mahaleb	5530	23137	346	103	496	41	113	4	nd
18	Pretzel, salted	5570	23304	342	62	517	59	111	4	nd
19	Chocolate, gluten-free	4960	20752	230	120	650	290	57	4	17
20	Tahini, gluten-free	5490	22970	331.4	nd	514.2	nd	98.1	nd	nd
21	Coconut, walnut, high fibre, gluten-free	4830	20208	235	156	614	268	31	4	70
22	Chocolate, gluten-free	5150	21547	264	133	660	202	22	6	nd

nd – not detected.

Statistical analysis

Statistical evaluation of HMF and MDA analyses was performed to determine the reliability of the data. The normal distribution of the data was checked by the Shapiro-Wilk test. Mean and standard deviation (SD) were calculated for normally distributed data, and differences between groups were analysed by one-way analysis of variance (ANOVA). In cases where the ANOVA test showed significant differences, Tukey's HSD (honest significant difference) test was used to determine the differences between pairs. Results with a p value below 0.05 were considered statistically significant. The section should provide information sufficient to enable reproduction of the experimental work.

RESULTS AND DISCUSSION

The HPLC chromatograms of MDA and HMF in cookies with tahini (sample 14) are presented in Fig. 1 and Fig. 2, respectively. The declared nutrient composition on the label, including carbohydrate, sugar, protein, saturated fat, total fat, fibre, and salt, in cookie samples, is given in Tab. 1. The energy content of 22 different cookie samples varied between 4 470–5 760 kcal·kg^{-1} (18 702–24 099 kJ·kg^{-1}), total fat content was between 200–401 g·kg^{-1} and sugar content ranged between 41–544 g·kg^{-1}. The high energy, fat, and sugar content may offer favourable environments for MDA and HMF formation in cookies.

MDA amounts of biscuit samples were compared before and after digestion, and bioaccessibility was presented in Tab. 2. Initial MDA values ranged from 0.17 ± 0.01 mg·kg^{-1} to 1.01 ± 0.03 mg·kg^{-1}. The highest initial value belongs to gluten-free tahini cookies (sample 20). Tahini, as a fatty ingredient, may be particularly prone to oxidation, which may facilitate the formation of MDA. In sample 21, gluten-free cookies with coconut, walnuts, and high fibre may be associated with lower MDA levels. Ingredients containing healthy fats such as coconut and walnuts are more resistant to oxidation and are fortified with natural antioxidants. The high fibre content may also inhibit oxidation processes, reducing MDA formation. Differences between products can be attributed to a number of factors, such as the properties of the ingredients used, cooking techniques, the stability of ingredients and oxidation processes.

After digestion, MDA levels ranged from 0.05 ± 0.00 mg·kg^{-1} to 0.61 ± 0.03 mg·kg^{-1}; bioaccessibility ranged between 17 ± 1 % and 329 ± 10 %. In general, after digestion, MDA levels decreased compared to baseline values, suggesting that MDA is degraded during digestion or absorption rates are low. However, in some samples (21, 22), after digestion, MDA values increased and bioaccessibility rates were found to be quite high. Especially in sample 21, the formation rate reached 329 ± 10 %, which may indicate that MDA became more bioavailable during digestion. High bioaccessibility levels suggest that MDA may be further absorbed in the intestine and therefore may cause potential oxidative stress in the body.

Increased in vitro MDA levels in gluten-free products (samples 21 and 22) may be due to the types of flour and starch usually used, which are more prone to oxidation. Gluten-free flours contain processed starches that may accelerate the oxidation process [17]. In addition, some gluten-free products contain high levels of saturated fats, which can make them more susceptible to oxidation [18]. Gluten-free products may also be poor in antioxidants, which can lead to increased

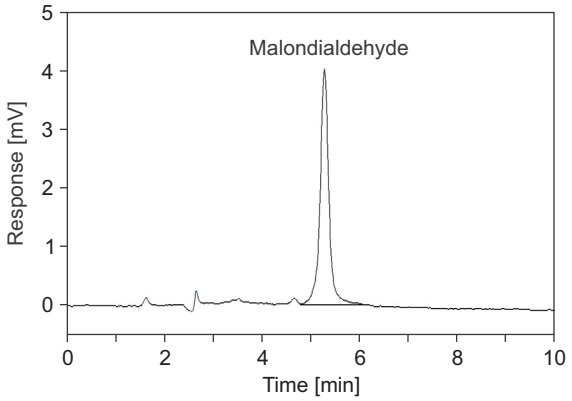

Fig. 1. The HPLC chromatogram of malondialdehyde in cookies with tahini (sample 14).

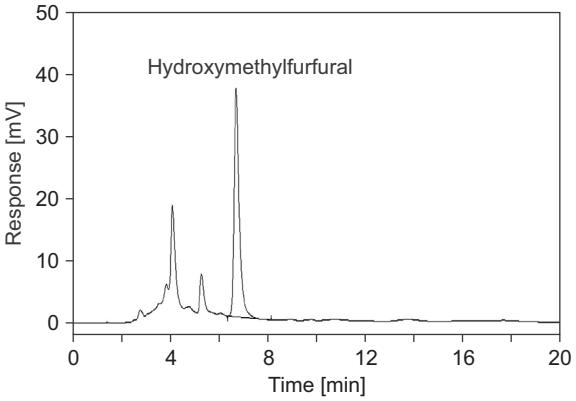

Fig. 2. The HPLC chromatogram of hydroxymethylfurfural in cookies with tahini (sample 14).

Tab. 2. Malondialdehyde values of the cookie samples and bioaccessibility.

No	Cookie	Malondialdehyde [mg·kg^{-1}]		Bioaccessibility [%]
		Initial value	After *in vitro* digestion	
1	Coconut, chocolate chips	0.42 ± 0.02^{a}	0.15 ± 0.01^{b}	36 ± 2
2	Gum drops, powdered sugar	0.66 ± 0.02^{a}	0.16 ± 0.01^{b}	24 ± 2
3	Butter	0.35 ± 0.02^{a}	0.14 ± 0.01^{b}	40 ± 4
4	Butter	0.19 ± 0.01^{a}	0.18 ± 0.01^{b}	95 ± 6
5	High fibre, raspberry, apple, hazelnut, no added sugar	0.53 ± 0.03^{a}	0.30 ± 0.02^{b}	57 ± 3
6	Chocolate chips	0.29 ± 0.01^{a}	0.05 ± 0.00^{b}	17 ± 1
7	Lemon, white chocolate	0.32 ± 0.02^{a}	0.19 ± 0.02^{b}	59 ± 5
8	Tahini, crispy	0.31 ± 0.02^{a}	0.16 ± 0.01^{b}	52 ± 2
9	Gum drops	0.43 ± 0.03^{a}	0.34 ± 0.01^{b}	80 ± 2
10	Kavala	0.65 ± 0.03^{a}	0.49 ± 0.01^{b}	75 ± 2
11	Chocolate	0.29 ± 0.01^{a}	0.14 ± 0.02^{b}	48 ± 5
12	Blueberry, cranberry	0.28 ± 0.02^{a}	0.05 ± 0.01^{b}	18 ± 2
13	White chocolate, cranberry	0.69 ± 0.04^{a}	0.25 ± 0.02^{b}	36 ± 2
14	Tahini	0.43 ± 0.03^{a}	0.13 ± 0.01^{b}	30 ± 3
15	Tahini, nut	0.41 ± 0.01^{a}	0.32 ± 0.02^{b}	78 ± 4
16	Nut, bitter almond	0.52 ± 0.02^{a}	0.17 ± 0.02^{b}	33 ± 4
17	Sunflower seed, mahaleb	0.51 ± 0.02^{a}	0.42 ± 0.03^{b}	82 ± 5
18	Pretzel, salted	0.33 ± 0.02^{a}	0.19 ± 0.01^{b}	58 ± 4
19	Chocolate, gluten-free	0.35 ± 0.02^{a}	0.20 ± 0.02^{b}	57 ± 6
20	Tahini, gluten-free	1.01 ± 0.03^{a}	0.41 ± 0.02^{b}	41 ± 2
21	Coconut, walnut, high fibre, gluten-free	0.17 ± 0.01^{a}	0.56 ± 0.02^{b}	329 ± 10
22	Chocolate, gluten-free	0.32 ± 0.02^{a}	0.61 ± 0.03^{b}	191 ± 9

Values are mean ± standard deviation (n = 3).

MDA levels. Furthermore, the additives and stabilisers used can also increase oxidation, leading to elevated MDA levels. Due to the absence of gluten protein in gluten-free products, lipids are freer and more easily oxidised during digestion [19]. Sample 21 may also be more prone to oxidation in relation to its contents of unsaturated fats, such as coconut and walnuts. On the contrary, at the beginning of the study, fruit-containing products (samples 5, 12, and 13) had high initial MDA levels; however, the bioaccessibility of MDA decreased after digestion. These results may be attributed to the antioxidant polyphenols of raspberry, blueberry and cranberry, which may suppress MDA production to some extent during digestion [20].

MDA is a small, reactive aldehyde molecule that is formed as a result of lipid oxidation and can interact with macromolecules such as cell membranes and DNA. MDA levels can increase or decrease depending on the rate of lipid oxidation during digestion. When lipid oxidation increases, the presence of free radicals and oxygen boosts MDA production, while conditions such as antioxidants and low pH can inhibit oxidation and reduce levels. Therefore, lipid content, the presence of oxidants and antioxidants, pH, and oxygen levels determine MDA levels [21]. It has been reported that lipid oxidation increases during the digestion of foods high in lipids, such as fish, meat, and fish oil, and that their levels rise as a result. In vitro digestion models and various studies have reported an increase in these oxidative compounds, particularly in the duodenal and intestinal phases [14, 22, 23]. During digestion, the presence of free radicals and oxygen, especially in the intestinal environment, leads to the oxidation of lipids, particularly unsaturated fatty acids. This process is accelerated by digestive conditions such as pH changes, enzyme activity, and temperature. Lipid oxidation causes the formation of peroxides and unstable aldehydes as free radicals attack the double bonds of lipids. Foods with high lipid content, especially fatty fish and red meat, have the potential to pro-

duce more oxidative products. Oxidation typically progresses through chain reactions, leading to small initial events accumulating over time to form significant oxidative loads. As a result, during lipid digestion, environmental factors and unsaturated bonds in lipid structure cause levels of oxidative products like MDA to increase [2].

Along with this, some studies have also reported decreased MDA levels in fish and seafood, meat and meat products, dairy products, and vegetable oils during digestion, particularly in the intestinal environment [14, 22, 24, 25]. Similar to all these studies, the present study also found a decrease in MDA levels after in vitro digestion. The decrease in MDA levels during digestion is due to MDA reacting with amino acids and proteins to form stable adducts or larger molecular structures. In addition, the amount of MDA in its free form may decrease through chemical reactions such as aldol condensation and hydrolysis. Changes in pH and enzymatic effects in the digestive environment affect the rate and direction of these reactions, reducing the proportion of MDA that remains in its free form. Furthermore, the formation of complexes between MDA and other reactive compounds also reduces the amount of free MDA and contributes to the control of lipid oxidation. All of these mechanisms cause a decrease in the levels of MDA in its free form during digestion [8, 22, 24].

HMF contents of cookie samples and HMF bioaccessibility levels after in vitro digestion were presented in Tab. 3. Initial HMF values ranged from 0.9 mg·kg^{-1} to 175.1 mg·kg^{-1} among the samples, with the highest values observed in cookies with white chocolate and cranberry (sample 13), and cookies with tahini (sample 14). In sample 13, the high sugar content and in sample 14, the high fat content in tahini may offer favourable environments for HMF formation during heat treatment, resulting in the highest HMF levels in these products.

Post-digestion HMF values ranged between 0.0 mg·kg^{-1} and 59.7 mg·kg^{-1}. The HMF bio-

Tab. 3. Hydroxymethylfurfural values of the cookie samples and bioaccessibility.

No	Cookie	Hydroxymethylfurfural [mg·kg^{-1}]		Bioaccessibility [%]
		Initial value	After *in vitro* digestion	
1	Coconut, chocolate chips	27.3 ± 1.5^{a}	4.3 ± 0.1^{b}	16 ± 0
2	Gum drops, powdered sugar	1.4 ± 0.1^{a}	1.1 ± 0.1^{b}	79 ± 5
3	Butter	9.9 ± 0.2^{a}	2.1 ± 0.1^{b}	21 ± 1
4	Butter	1.6 ± 0.1^{a}	0.0 ± 0.0^{b}	0 ± 0
5	High fibre, raspberry, apple, hazelnut, no added sugar	63.5 ± 2.3^{a}	56.1 ± 2.7^{b}	88 ± 4
6	Chocolate chips	2.1 ± 0.1^{a}	0.6 ± 0.0^{b}	29 ± 2
7	Lemon, white chocolate	0.9 ± 0.0^{a}	1.8 ± 0.1^{b}	200 ± 8
8	Tahini, crispy	14.6 ± 0.6^{a}	3.6 ± 0.2^{b}	25 ± 1
9	Gum drops	12.3 ± 0.6^{a}	2.5 ± 0.1^{b}	20 ± 1
10	Kavala	20.1 ± 0.8^{a}	18.5 ± 1.1^{b}	92 ± 6
11	Chocolate	6.7 ± 0.2^{a}	0.6 ± 0.0^{b}	9 ± 1
12	Blueberry, cranberry	15.5 ± 0.6^{a}	6.5 ± 0.2^{b}	42 ± 1
13	White chocolate, cranberry	175.1 ± 10.2^{a}	59.7 ± 1.4^{b}	34 ± 1
14	Tahini	118.8 ± 8.3^{a}	4.1 ± 0.1^{b}	4 ± 0
15	Tahini, nut	56.5 ± 1.0^{a}	2.1 ± 0.1^{b}	4 ± 0
16	Nut, bitter almond	3.8 ± 0.1^{a}	0.1 ± 0.0^{b}	3 ± 0
17	Sunflower seed, mahaleb	7.8 ± 0.1^{a}	4.7 ± 0.1^{b}	60 ± 2
18	Pretzel, salted	2.0 ± 0.1^{a}	0.6 ± 0.1^{b}	30 ± 3
19	Chocolate, gluten-free	3.5 ± 0.1^{a}	0.1 ± 0.0^{b}	3 ± 0
20	Tahini, gluten-free	10.7 ± 0.6^{a}	0.8 ± 0.0^{b}	8 ± 0
21	Coconut, walnut, high fibre, gluten-free	2.1 ± 0.1^{a}	2.3 ± 0.1^{b}	110 ± 5
22	Chocolate, gluten-free	4.2 ± 0.1^{a}	5.0 ± 0.1^{b}	119 ± 2

Values are mean ± standard deviation (n = 3).

accessibility ratios of the samples varied from 0 ± 0 % to 200 ± 8 %; it was noteworthy that the amount of HMF after digestion in some samples (samples 7, 21 and 22) was above the initial value. This suggests the possibility that HMF may regenerate in the digestion medium or that HMF bound in the previous matrix structure may become free. However, since the amount of HMF in these products is low at the beginning, even if the bioaccessibility is high, the amount of HMF that can be absorbed is limited; therefore, post-digestion HMF levels have generally remained low among the products. Both initial and post-digestion values of HMF were high in samples 5 and 13, while very low bioaccessibility values were reported in samples 4, 14, 15, 16 and 19. These results indicate that the HMF content of biscuit products is highly variable and bioaccessibility may vary in relation to the composition, production conditions, and matrix structure of the product. This situation suggests the possibility that HMF may be reformed in the digestive environment or that HMF bound in the previous matrix structure may be released.

HMF is a carbonyl compound formed during the drying of sugars in an acidic environment at high temperatures as a result of the Maillard reaction, and it can be found in large quantities in foods exposed to high heat processing. The change in the amount of HMF during digestion is closely related to its formation and reactions, and these conditions are important for health. In this context, it is important to investigate HMF formation under digestive system conditions.

Several studies have reported an increase in HMF levels during digestion in breakfast cereals, noodles, potato crips and biscuits [26–28]. There are several fundamental reasons for the increase in HMF during digestion. Firstly, the proteolysis process and the formation of intermediate products, such as 3-deoxyglucosone and Schiff bases, play an important role in this increase. Additionally, the specific pH and temperature conditions of the digestion environment facilitate the conversion of existing compounds into HMF by increasing reaction rates. HMF can also react with amino acids and proteins to form new HMF derivatives. However, HMF formed during cooking can be released from the food matrix and undergo further transformations during digestion. These processes contribute to the increase in HMF levels through the transformation and release of existing precursor compounds rather than the synthesis of HMF from scratch [8]. On the contrary, a decrease in HMF levels has also been reported in biscuits, noodles, potato, meat and fish [26, 28, 29]. Hamzalioğlu and Gökmen [26] have reported that the initial HMF content in various biscuit samples ranged from approximately 3.86 mg·kg^{-1} to 55.88 mg·kg^{-1}. However, at the end of digestion, HMF levels were found to be from approximately 40.3 mg·kg^{-1} (in regular biscuits) to 70.76 mg·kg^{-1} (in twice-baked biscuits). Similar to these findings, HMF levels decreased following in vitro digestion in the present study. In particular, during the gastric phase, HMF levels may increase due to the conversion of intermediate products formed during cooking (such as sugar derivatives like 3-deoxyglucosone and 3,4-deoxyglucosone) to HMF in an acidic environment. However, in the later stages of digestion, particularly in the intestinal phase, the aldehyde groups in the structure of HMF react with amino acids and sulfhydryl groups, leading to chemical processes such as Michael addition and Schiff base formation. Therefore, the behaviour of HMF during digestion depends on the dynamic balance between formation and degradation reactions. The main reason for the decrease in HMF observed during digestion is that HMF reacts with proteins, especially amino acids, to form adducts [26].

Briefly, cookies may pose a potential risk in terms of MDA and HMF since they are prepared by baking ingredients containing high levels of sugar and amino acids at high temperatures. In particular, toxic compounds such as HMF and MDA, which are formed as a result of lipid oxidation, are produced in cookies during caramelisation and Maillard reactions. These reactions are triggered by high heat and long baking times, while HMF levels also increase due to the conversion of intermediary substances. The intake of these compounds can cause cellular damage and increase the risk of chronic diseases through lipid peroxidation and genotoxic effects [30]. Therefore, as cookies are exposed to high heat and prolonged baking, MDA and HMF levels rise, increasing potential health risks. This study also presented important results in terms of revealing the effects of in vitro digestion in the small intestine. However, after digestion in the small intestine, MDA and HMF can reach the colon, where they can interact with the gut microbiota. MDA and HMF can be metabolised by microbial activity in the colon environment, affecting the formation of short-chain fatty acids (SCFA). SCFA production in the colon provides numerous health benefits, including inhibition of pathogenic bacteria, protection of the intestinal barrier, protective effects against obesity, and reduced risk of colorectal cancer [31]. Considering all these protective effects, future studies should focus on the interactions of MDA

and HMF with the gut microbiome and their roles in SCFA formation; however, this topic is beyond the scope of the current study.

CONCLUSIONS

In conclusion, the present study revealed that cookies may contain significant amounts of MDA and HMF which may depend on factors such as baking temperature and time, sugar and amino acid content, lipid types, storage conditions, and additives. The highest levels of MDA and HMF in cookies were found in types containing white chocolate and tahini. This study also showed that in vitro digestive system conditions may decrease the formation of MDA and HMF in most of the cookie samples. On the contrary, the bioaccessibility of MDA and HMF was strongly increased in gluten-free cookies. These results may be related to the acceleration of the oxidation process of processed starch used in gluten-free products, the saturated fat content of gluten-free products, insufficient antioxidants, high sugar content, lipid oxidation, additives, and the continuation of chemical reactions due to different content structures. In summary, this study has shown that cookies may contain high HMF and MDA levels, and that these amounts may increase or decrease depending on conditions in the digestive system and the food matrix. Future studies should also focus on the interactions between the colon and MDA and HMF.

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