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ORIGINAL PAPER

Comparative analysis of hydroxymethylfurfural and malondialdehyde bioaccessibility in biscuits with varied dietary fiber content

Halime Uğur[1] · Elif Ede-Cintesun[2] · Jale Çatak[2] · Yavuz Beceren[2] · Murat Gürbüz[3] · Mustafa Yaman[4,5]

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Abstract

Hydroxymethylfurfural (HMF) and malondialdehyde (MDA) are heat-induced toxic compounds commonly formed in processed foods rich in carbohydrates and fat. Despite extensive studies reporting their occurrence in bakery products, limited information is available regarding their bioaccessibility under gastrointestinal digestion conditions, particularly in relation to dietary fiber content. The aim of this study was to determine the concentrations of HMF and MDA in diverse biscuit formulations under in vitro gastrointestinal digestion conditions, based on the assumption that differences in biscuit composition, particularly dietary fiber content, may influence both their initial levels and bioaccessibility during digestion. For that purpose, 24 different biscuit samples were purchased from commercial markets. HMF and MDA contents were determined at initial and after in vitro digestion. The initial values ranged from 67.0 to 4208.3 µg/100 g and 40.2 to 106.9 µg/100 g, for HMF and MDA, respectively. After digestion, statistically significant increases in the bioaccessibility of HMF and MDA were observed, reaching up to 495.4% and 361.8%, respectively ($p < 0.05$). High-fiber non-light biscuits exhibited the lowest initial HMF levels despite having a higher sugar content. The high carbohydrate content resulted in elevated HMF and MDA levels following digestion. The high-fat biscuits exhibited unexpectedly lower MDA levels post-digestion, which was attributed to their lower initial MDA levels. Further research is required to elucidate the impact of food components on HMF and MDA bioaccessibility.

Highlights

- High fibre biscuits had lower initial HMF levels than low fibre biscuits.
- The lowest initial HMF levels were observed in high fibre non-light biscuits.
- Bioaccessibility of HMF and MDA were increased under in vitro digestion.
- High fibre light-biscuits had the highest initial MDA levels, possibly due to high PUFA content .

Keywords Biscuit · Hydroxymethylfurfural · Malondialdehyde · *in vitro* · Bioaccesibility

✉ Halime Uğur
halime.halimeugur@gmail.com

Elif Ede-Cintesun
elif.ede@izu.edu.tr

Jale Çatak
jalecatak@gmail.com

Yavuz Beceren
yavuzselimbeceren@gmail.com

Murat Gürbüz
muratgurbuz@ohu.edu.tr

Mustafa Yaman
mustafayaman1977@gmail.com

[1] Department of Nutrition and Dietetics, Faculty of Health Sciences, Istanbul Medeniyet University, Istanbul, Türkiye

[2] Department of Nutrition and Dietetics, Faculty of Health Sciences, Istanbul Sabahattin Zaim University, Istanbul, Türkiye

[3] Department of Nutrition and Dietetics, Faculty of Health Sciences, Niğde Ömer Halisdemir University, Niğde, Türkiye

[4] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Istanbul Sabahattin Zaim University, Istanbul, Türkiye

[5] Sabri Ülker Food and Nutrition R&D Center, Istanbul Sabahattin Zaim University, Istanbul, Turkey

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Introduction

Procedures such as heat, sterilization, and evaporation applied in food production processes can result in the formation of Maillard reaction products and changes in food quality. Compounds such as furan and furfural, which are products of this reaction, are worrisome when they appear in the production chain as they can cause potential health problems [1]. Maillard reaction, which creates a pleasant color, taste, texture, and flavor in bakery products during baking, also creates potentially cytotoxic, mutagenic, and genotoxic compounds such as hydroxymethylfurfural [2]. Studies on processed foods have focused on four compounds, such as furfural (F), 5-methyl-2-furfural (MF), 2-acetylfuran (FMC), and 5-hydroxymethyl-2-furfural (5-HMF) due to their toxicity. 5-HMF can be used as a potential indicator to assess the level of heat treatment during food processing [1, 3]. However, 5-HMF is known to be toxic; its adverse effect on human health only occurs in the presence of high concentrations [4]. Although most of the studies about 5 HMF have focused on carbohydrate-rich foods such as bakery products, potato derivatives, honey, jam, infant formulas, breakfast cereals, cookies, and cakes, 5-HMF has also been found in aged vinegar, coffee, milk, heat-treated roasted squid, and chicken [1, 5, 6]. Malondialdehyde (MDA), another toxic compound, is a three-carbon dialdehyde formed as a result of the peroxidation of n-6 and n-3 PUFAs. Globally, it has been used for many years as an important lipid oxidation marker [7]. This highly reactive carbonyl compound also affects important macromolecules such as proteins or DNA. Because MDA reacts very easily with amino groups, which cause modification of these macromolecules [8]. Although MDA limit levels are not officially stated, the European Food Safety Authority scientific committee has specified 30 µg/kg body weight/day as the threshold for toxicological concern in terms of exposure levels [7]. Exposure to heat treatment and storage of various foods for a long time causes the formation of both HMF and MDA, as shown in Fig. 1a and b [4].

However, these parameters, which play an important role in the formation of HMF and MDA, are also the most important processes for the quality of biscuits, which are an important component of the food industry [9].

The food industry is gradually interested in the development of innovative food products that are beneficial to health, functional, or taste more pleasing [10]. In this context, attempts are made to change and improve the composition of biscuits, which have a significant global consumption level, to increase their nutritional values and functionality [11]. Biscuit is one of the flour-based ready-to-eat snacks preferred by all age groups in many countries due to its ease of purchase, accessibility, long shelf life, and pleasant taste [12]. Biscuit dough generally contains common ingredients such as flour, water, sugar,

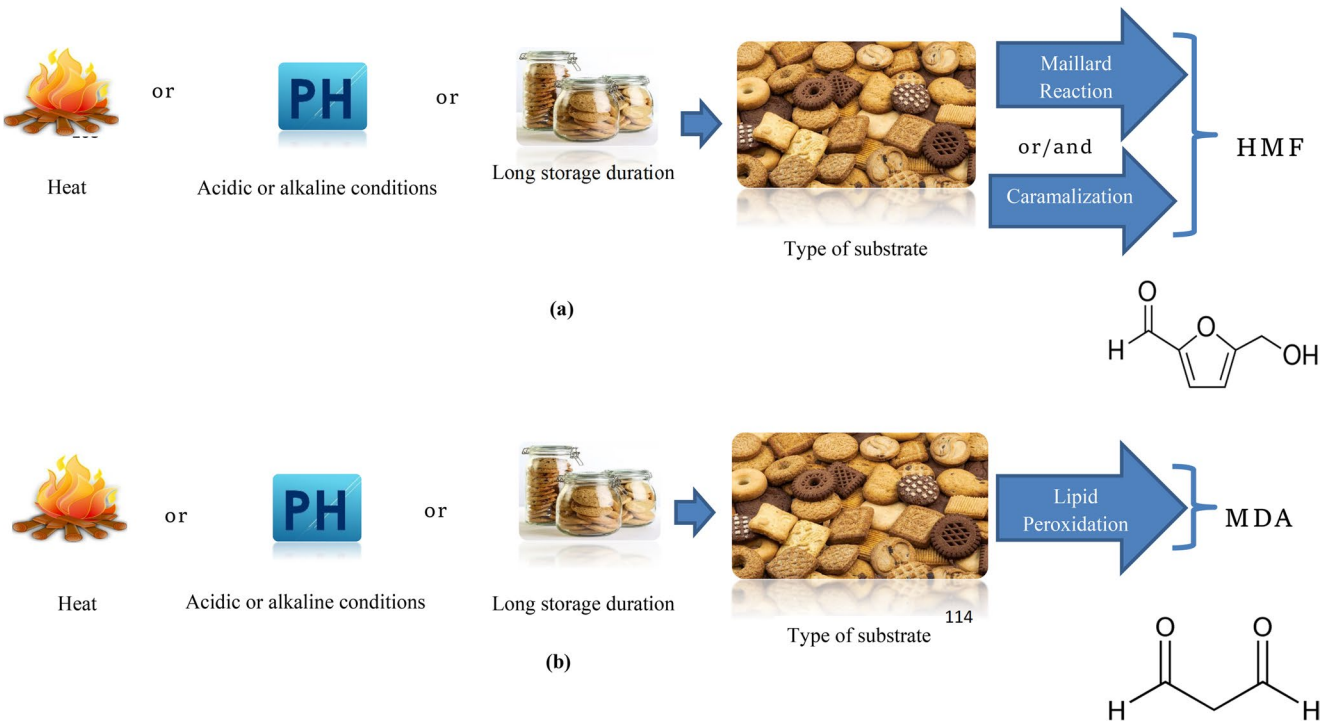


Fig. 1 Formation pathways of (**a**) 5-hydroxymethyl-2-furfural (HMF) and (**b**) malondialdehyde (MDA) in biscuits

and fat, as well as different ingredients depending on the type (fiber, wheat-rice bran, milk powder, baking powder, vanillin extract, specific aroma, etc.) [13, 14]. The amount of easily digestible carbohydrates that raise blood sugar quickly, and the fat and generally low fiber content of biscuits, preclude them from being viewed as a healthy snack [13]. One of the public health approaches is to increase the amount of fiber consumed daily. Biscuits, one of the industrial products, are also important candidates for the addition of dietary fiber [13, 15]. In the food industry, many different types of fiber are used as emulsifiers, stabilizers, and thickening agents, and to create low-calorie foods. In some studies, it has been shown that fibers can interact with acrylamide as well as organic and inorganic contaminants and restrict the bioaccessibility of these substances [16].

Determining the amount of HMF and MDA, which are oxidation products that have the potential to cause many chronic diseases, in different types of biscuits may be important for developing strategies to reduce dietary exposure to these components. Additionally, HMF and MDA concenterations may vary depending on digestive tract conditions. To our knowledge, studies focused on the effects of digestion on the formation of MDA and HMF in such foods are extremely limited. Therefore, the aim of our study is to investigate the effects of different food matrices and digestion processes on the amount and bioaccessibility of HMF and MDA in biscuits sold most in Türkiye.

Materials and methods

Samples

A total of 24 different creamless biscuit samples were purchased from common commercial markets in Istanbul, Türkiye. The energy and nutrient contents of the creamless biscuits are shown in Table 1. The sample size was selected to represent a broad range of commercially available biscuit brands with different nutritional compositions, appropriate for an in vitro comparative study. All analyses were performed in triplicate to ensure analytical reliability and reproducibility.

Reagents and materials

5-(hydroxymethyl) furfural (99%), Trichloroacetic acid (TCA), 1,1,3,3-Tetraethoxypropane (≥96%), Potassium dihydrogen phosphate (KH_2PO_4) (99.5% purity Merck), 2-Thiobarbituric acid, methanol (HPLC grade), acetonitrile (HPLC grade) and other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Table 1 Energy and nutrient composition in some creamless biscuits

	Sample Name	Total energy (kcal)	Total fat (g)	Saturated fat (g)	Trans fat (g)	Carbohydrates (g)	Sugars (g)	Dietary fiber (g)	Protein (g)	Salt (g)
High fiber content creamless biscuits (non-light) (100 g)	1	455	18.0	9.5	0.0	62.0	20.0	5.8	8.0	1.1
	2	486	22.2	16.8	0.0	61.1	27.6	8.0	5.2	0.9
	3	453	18.0	8.6	-	64.0	19.0	5.5	6.5	1.5
	4	470	19.0	10.6	0.0	62.4	20.4	5.7	9.8	0.7
	5	458	18.0	9.7	0.0	63.0	22.0	5.4	7.5	1.0
	6	464	19.0	10.0	0.2	65.0	24.0	6.0	6.6	1.1
	7	462	20.0	9.5	-	59.0	11.0	7.7	8.5	3.0
	8	477	22.0	10.0	-	58.0	11.0	5.2	9.4	2.7
High fiber content creamless biscuits (light) (100 g)	9	422	11.0	5.5	-	62.0	4.8	11.0	13	2.6
	10	445	14.0	6.9	-	70.0	9.8	5.2	7.8	0.26
	11	405	9.5	4.1	-	63.0	0.7	11.0	11	1.2
	12	414	12.0	5.7	0.0	61.0	7.5	8.0	12	2.8
	13	417	11.0	4.8	-	67.0	14.0	8.4	8.6	0.47
	14	409	11.0	4.4	-	64.0	3.2	8.5	10	2.0
	15	409	11.0	4.5	0.0	61.0	5.3	9.6	12	2.3
	16	400	9.8	4.6	-	56.0	6.5	11.0	16	1.8
Low fiber content creamless biscuits (non-light) (100 g)	17	456	20.0	9.6	-	61.0	7.3	1.8	7.7	3.0
	18	482	21.0	9.0	-	65.0	24.0	1.9	7.1	0.96
	19	470	19.0	9.0	-	66.0	20.0	3.0	8.0	0.84
	20	456	20.0	9.3	-	60.0	7.6	2.2	7.6	2.9
	21	463	16.0	7.7	-	71.0	24.0	1.7	7.5	0.81
	22	440	12.0	5.7	0.1	73.7	16.0	2.8	8.0	0.66
	23	439	13.0	6.3	0.1	70.3	19.2	3.9	3.2	0.8
	24	445	14.4	6.7	0.1	67.9	16.8	2.6	9.7	0.8

HMF analysis with HPLC

The sample preparation method and HPLC parameters described by Ameur et al. (2006) [17] were adapted with minor modifications. The extraction was carried out using TCA, as outlined in the MDA analysis. After extraction, the solution was filtered through a 0.45 µm CA filter before being injected into the HPLC system.

The HPLC setup included a Shimadzu LC-20AT pump coupled with a Shimadzu SPD-20 A UV/VIS detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase consisted of water and methanol in an 80:20 (v/v) ratio. The mobile phase consisted of water and methanol in an 80:20 (v/v) ratio and was operated under isocratic conditions. A Gemini-NX 5µ C18 110Å, 4.6 mm × 250 mm column was utilized for HMF separation. The analysis was conducted at a flow rate of 0.5 mL/min, with the column oven maintained at 25 °C.

HMF was quantified using an external standard method. A five-point calibration curve (1–100 µg/mL) was prepared, and peak areas were measured in triplicate. Linearity was confirmed across this range.

MDA analysis with HPLC

The method described by Gürbüz et al. [4] was adapted with minor modifications. Initially, a 2 g biscuit sample was placed into a 50 mL Falcon tube, and 2 mL of 20% TCA solution was added. The mixture was then centrifuged at 8000 rpm for 10 min. The resulting supernatant was also utilized for HMF analysis. Afterward, 1 mL of the centrifuged liquid phase was combined with 1 mL of thiobarbituric acid solution (6.7 mg/mL) for derivatization and incubated in a hot water bath at 90 °C for 30 min. Once cooled to room temperature, the solution was filtered using a 0.45 µm CA filter before HPLC injection.

MDA analysis was performed using a Shimadzu Nexera-i HPLC system equipped with a Shimadzu RF-20 A fluorescence detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase consisted of a 0.05 M KH_2PO_4 buffer solution, methanol, and acetonitrile in a 72:17:11 ratio and was operated under isocratic conditions. Separation was achieved using a Gemini-NX 5µ C18 110Å, 4.6 mm × 250 mm HPLC column. The excitation and emission wavelengths were set at 530 nm and 550 nm, respectively. The column oven was maintained at 25 °C, with a flow rate of 1 mL/min.

MDA was quantified using an external standard method after derivatization. A five-point calibration curve (1–20 µg/mL) was prepared, and peak areas were measured in triplicate. Linearity was confirmed across this range.

Separate HPLC methods were employed for HMF and MDA due to their different chemical properties and detection requirements. HMF was quantified using a UV detector, whereas MDA, derivatized with thiobarbituric acid to form a fluorescent adduct, required a fluorescence detector. Therefore, different mobile phases and detection systems were used for accurate quantification of each compound.

In vitro digestion and bioaccessibility of HMF and MDA

The in vitro study was conducted following the methodology outlined by the standardized INFOGEST static digestion protocol [18, 19] with certain modifications. The protocol consisted of sequential oral, gastric, and intestinal phases under controlled pH, enzyme activities, and incubation conditions. A schematic overview of the digestion procedure is provided in the Supplementary Material (Fig. S1). Figure S1 provides an overview of the preparation of the in vitro digestion environment and the digestion procedure. Saliva solution, gastric fluid, duodenal fluid, and bile fluid were prepared using a combination of organic and inorganic compounds along with digestive enzymes. Each organic and inorganic component was dissolved in 1000 mL of distilled water for the respective digestive enzyme in this in vitro human digestion model. The enzymes were then incorporated into the solutions and thoroughly mixed. The pH was adjusted to the appropriate level for each solution using 1 M HCl or 0.2 M NaOH, as specified in Figure S1.

During the oral phase, a 5 g homogenized biscuit test sample was placed in a 50 mL Falcon tube along with 5 mL of saliva solution (solid-to-fluid ratio, 1:1, w/v), then vortexed for 20 s. The mixture was incubated at 37 °C in a shaking water bath for 5 min before proceeding to the gastric phase. In this step, the sample from the oral phase was mixed with 12 mL of gastric juice and incubated at 37 °C in a shaking water bath for 2 h. Next, the sample from the gastric phase was combined with 10 mL of duodenal fluid and 5 mL of bile solution, followed by incubation in a shaking water bath at 37 °C for another 2 h.

After digestion was completed, the pH of the solution was adjusted to 4.5 using TCA. The mixture was then diluted to 50 mL with deionized water and centrifuged at 8000 rpm for 10 min. The resulting supernatant was used for MDA analysis.

Statistical analysis

All analyses were performed in triplicate, and the average values were used for evaluation. A one-way analysis of variance (ANOVA) was conducted ($p < 0.05$) using Tukey's test to assess significant differences statistically.

Results and discussion

The HPLC chromatograms of MDA and HMF in high fiber content creamless non-light biscuits are shown in Figs. 2 and 3 (samples 1, respectively).

The declared amount of macronutrients including fat, carbohydrate, sugar, fiber, protein, and salt of samples are given in Table 1. As given in Table 1, high fiber non-light, high fiber light, and low fiber non-light biscuit samples consist of high levels of carbohydrates ranging from 58.0 to 65.0 g/100 g, 56.0 to 70.0 g/100 g, and 60.0 to 73.7 g/100 g, respectively. The sugar values range 11.0 to 27.6 g/100 g, 0.7 to 14.0 g/100 g, and 7.3 to 24.0 g/100 g, respectively, while, the fat values range from 18.0 to 22.2 g/100 g, 9.5 to 14.0 g/100 g, and 12.0 to 21.0 g/100 g, respectively. Besides, high fiber non-light and low fiber non-light biscuit samples have higher fat and sugar value ranges than high fiber light biscuit samples. When looking at the ranges of protein values, contrary to sugar and fat content, it is higher in high fiber light biscuit samples (7.8 to 16.0 g/100 g) compared to other groups (high fiber non-light: 5.2 to 9.8 g/100 g and low fiber non-light: 3.2 to 9.7 g/100 g). It is seen that these macronutrient compositions in biscuits are suitable for the formation of HMF, which is a product of the Maillard reaction between reducing sugars and compounds with free amino groups and is also formed as a result of caramelization. This suitable composition also applies to MDA, which is formed as a result of lipid oxidation [7, 20].

Dietary fibers have long been widely used in the food industry for the creation of food formulations, due to their swelling capacity, increasing viscosity, texture, or gel-forming properties as well as health-protecting and promoting properties [21, 22]. As given in Table 1, high fiber non-light biscuit samples and high fiber light biscuit samples contain high levels of dietary fiber ranging from 5.2 to 8.9 g/100 g and 5.2 to 11.0 g/100 g, respectively, while low fiber non-light biscuit samples contain lower levels of dietary fiber ranging from 1.7 to 3.9 g/100 g. Dietary fiber has been suggested to be associated with a reduced formation of oxidation products during cooking, potentially related to its water-holding and fat-binding properties. In addition, the industrial use of fiber as a partial substitute for flour, oil,

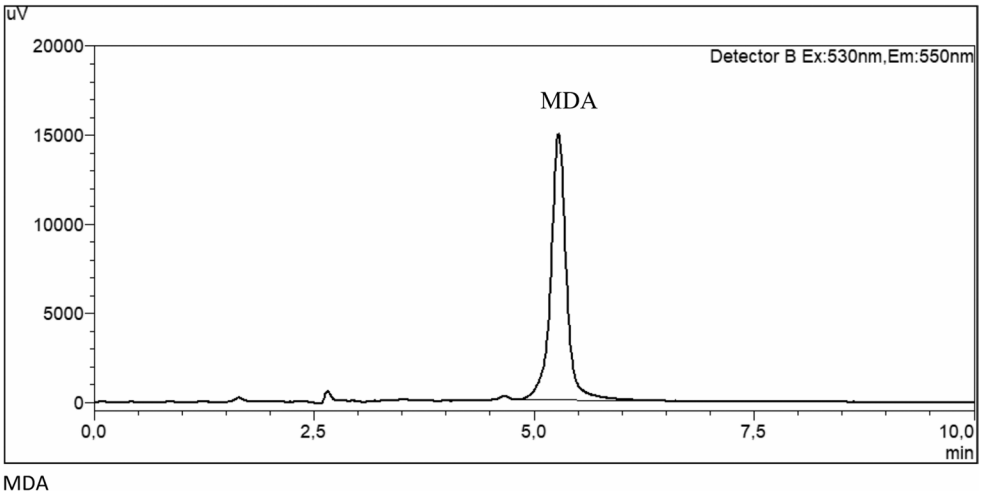


Fig. 2 The HPLC chromatogram of MDA in high fiber content creamless non-light biscuit

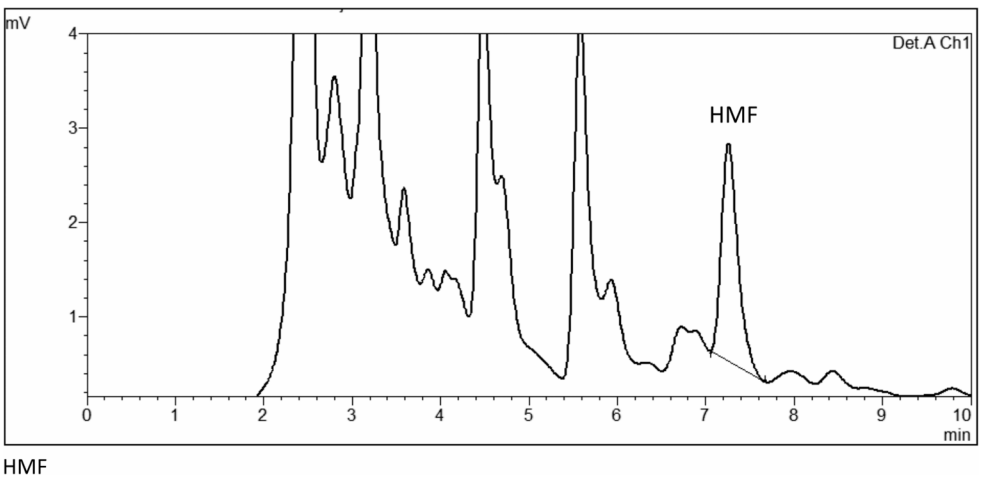


Fig. 3 The HPLC chromatogram of HMF in high fiber content creamless non-light biscuit

and sugar has been reported as an approach to limit oxidation [22, 23]. Consistent with this information, studies have shown that HMF and MDA formations are reduced in products enriched with fiber content [7, 24].

The measured HMF values of biscuits at initial and after digestion are presented in Table 2. Statistically significant differences ($p < 0.05$) were observed between initial and after-digestion HMF values for almost all biscuit samples, as shown in Table 2. In this study, the *initial value* refers to the concentration of HMF or MDA measured directly in the biscuit samples before digestion, whereas the *after digestion* value represents the amount detected following in vitro gastrointestinal digestion. *Bioaccessibility (%)* was calculated as the ratio of the concentration measured after digestion to the initial value and reflects the relative change in HMF and MDA levels under digestive conditions. Differences in initial HMF value ranges were observed among biscuit groups, with high fiber non-light biscuits generally characterized by lower ranges compared to high fiber light and low fiber non-light biscuits. During the baking process of the biscuit, caramelization and Maillard reaction occur, which causes the formation of HMF [2]. In this formation, properties such as heating, humidity, pH, NH_2, O_2, food composition (carbohydrate, protein, fat, sugar, glucose, fructose, fiber, phenolic compounds, etc.), cooking time, storage temperature, and water activity are the most important parameters [25, 26]. Moderate humidity, temperature above 50 °C, and pH 4–7 are optimum for the Maillard reaction, while caramelization requires stronger conditions such as low water activity, temperature higher than 120 °C, pH lower than 3 or higher than 9 [27]. In a study investigating the effects of sugar type, cooking time, and temperature on the development of HMF in cookies, HMF levels were found in the range of 4.5–74.9 mg/kg. In addition, in the same study, it was determined that the cookie samples with fructose sugar and baked at 170 °C for 20 min had the highest HMF levels [28]. In a related study, cookies with sucrose content baked at 200 °C had lower HMF levels, while cookies containing fructose and glucose were found to contain lower HMF levels when temperatures of 250 and 300 °C were reached. This is explained by the relative stability of sucrose at 200 °C [17]. Fang et al. (2022), in a study evaluating the formation of HMF in biscuits with sodium alginate, pectin, and chitosan added, reported that HMF was not detected in all of the biscuits, including the control group, except for the samples with 1.0% and 1.5% commercial pectin powder. It has been reported that this is due to the conversion of glucose carried by commercial citrus pectin to HMF [2]. The samples with the highest initial HMF levels were predominantly found in the low-fiber non-light biscuit group, whereas the lowest initial HMF levels were observed among high-fiber non-light biscuits. These differences were associated with variations in carbohydrate and dietary fiber contents among the samples (Table 2). When compared with the samples in the other group, it was seen that they had higher sugar content than most of them, which may partly be related to differences in sugar content.

Parameters such as components in the biscuit formulation, low humidity, high temperature, and short cooking time (up to 200 °C for less than 20 min) are the components of a complex process that reveals physical, chemical, and biochemical changes. HMF, which occurs as a result of Maillard reactions and caramelization triggered by these changes, has been reported in cereal products such as baby cereals, breakfast cereals, pasta, and bakery products in many studies [27]. The MDA values of high fiber non-light, high fiber light, and low fiber non-light biscuits at initial are presented in Table 3. For all biscuit samples, MDA levels after in vitro digestion were significantly higher than the initial values ($p < 0.05$), as indicated in Table 3. Differences in initial MDA value ranges were observed among biscuit groups. Overall, variability in MDA levels was evident across all groups, with no single biscuit group consistently

Table 2 Amount and bioaccessibility of HMF in some creamless biscuits initially and after in vitro digestion

	Sample Name	Initial value (µg/100 g)	After Digestion (µg/100 g)	Bioaccessibility (%)
High fiber content creamless biscuits (non-light) (100 g)	1	555.3 ± 16.2^a	740.0 ± 12.1^b	133.3 ± 2.9
	2	67.0 ± 4.0^a	115.0 ± 11.5^b	171.6 ± 17.6
	3	329.0 ± 9.0^a	142.0 ± 19.6^b	56.4 ± 39.1
	4	278.3 ± 8.7^a	315.0 ± 9.0^b	116.1 ± 4.0
	5	177.3 ± 7.1^a	549.3 ± 15.2^b	309.7 ± 9.2
	6	113.0 ± 4.0^a	209.6 ± 11.0^b	185.6 ± 10.0
	7	455.3 ± 11.2^a	525.0 ± 13.6^b	119.3 ± 2.0
	8	573.6 ± 8.1^a	765.0 ± 10.6^b	129.9 ± 1.1
High fiber content creamless biscuits (light) (100 g)	9	422.0 ± 11.3^a	587.0 ± 28.0^b	139.1 ± 6.6
	10	1253.0 ± 82.2^a	1177.3 ± 83.0^a	94.0 ± 6.6
	11	218.0 ± 10.5^a	1080.0 ± 78.0^b	495.4 ± 35.8
	12	754.6 ± 12.6^a	786.0 ± 10.5^b	104.2 ± 1.4
	13	2536.6 ± 167.7^a	3443.67 ± 103.9^b	135.8 ± 4.1
	14	1480.3 ± 138.3^a	1173.6 ± 99.5^b	79.3 ± 6.7
	15	350.3 ± 47.5^a	509.3 ± 20.0^b	145.4 ± 5.7
	16	1491.0 ± 69.5^a	1574.0 ± 111.0^b	105.6 ± 7.5
Low fiber content creamless biscuits (non-light) (100 g)	17	910.0 ± 24.3^a	1038.0 ± 76.0^b	114.1 ± 8.4
	18	530.6 ± 16.2^a	394.0 ± 8.5^b	74.3 ± 1.6
	19	2997.0 ± 180.2^a	3331.6 ± 101.1^b	111.2 ± 3.4
	20	536.0 ± 27.7^a	430.0 ± 14.2^b	80.2 ± 2.7
	21	931.0 ± 18.4^a	1342.3 ± 102.2^b	144.2 ± 11.0
	22	1343.6 ± 102.1^a	1745.3 ± 110.0^b	129.9 ± 8.2
	23	2551.3 ± 145.6^a	3022.3 ± 113.9^b	118.5 ± 4.5
	24	4208.3 ± 165.8^a	4621.0 ± 152.3^b	109.8 ± 3.6

Values are means ± range, $n = 3$. The different letters (a, b) in the same row indicate statistically significant differences between initial and after-digestion values for each biscuit sample (ANOVA, $p < 0.05$, Tukey's test)

exhibiting exclusively the highest or lowest initial MDA values. Biscuit groups associated with relatively higher initial MDA levels showed higher average protein, salt, and fiber contents, along with lower average sugar and total fat contents, compared to other groups. Although the total fat content was low, the proportion of unsaturated fatty acids in total fat (approximately 50–60%) was higher than that of saturated fatty acids. In comparison, the proportion of unsaturated fatty acids ranged between 51.5 and 57.2% in low-fiber non-light biscuits and between 24.3 and 55.0% in biscuits with relatively lower initial MDA levels. MDA caused by lipid oxidation that occurs during processing and storage of high-fat products is affected by the lipid content and composition of the product. An increase in the amount of lipids and the ratio between polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs) increases lipid oxidation and thus the amount of MDA [29, 30]. Zhuang et al. (2022), in a study examining the effect of heat treatments applied to soybean oil, palm oil, olive oil, and lard oil on the formation of lipid oxidation products (LOPs), LOPs were found to increase significantly with increasing temperature. In addition, in the same study, it was observed that the MDA content of soybean oil, which is rich in PUFA, was higher than other oils under 200 °C temperature conditions.

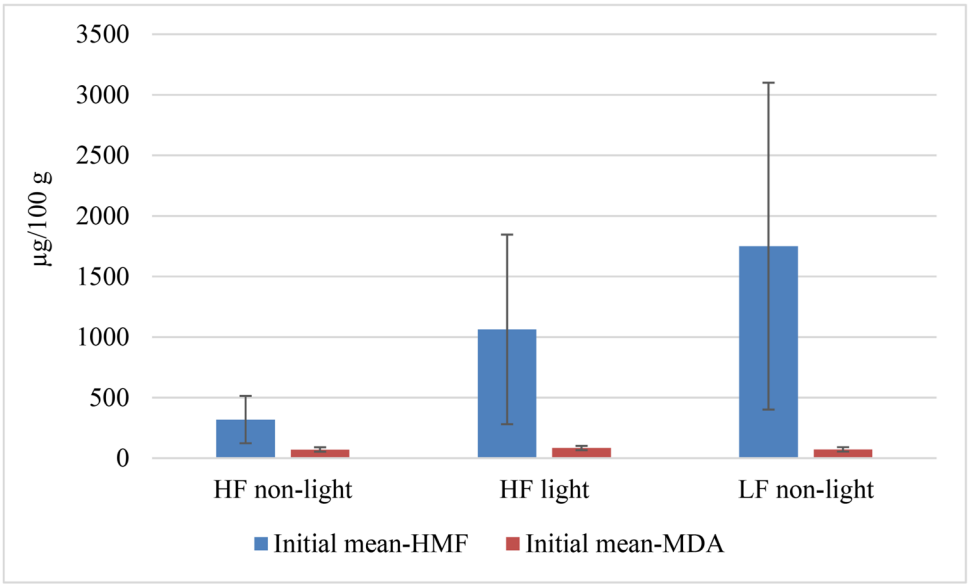
Therefore, it was stated that appropriately reducing the heating temperature of oils is important in preventing the formation of LOPs at high levels [31]. In a study conducted on 17 products, including vegetable oil, fried foods, baked foods, raw processed foods, and formula foods, which have a high consumption frequency in China, it was determined that cookies and Shortcrust pastry products contain lower levels of MDA compared to other heat-treated products (74 and 45 µg/100 g, respectively). It has been estimated that this is due to the fact that fatty foods are more likely to oxidize during heating [30]. Lamothe et al. (2019), in an in vitro study examining the effect of casein and whey proteins on the oxidation of flaxseed oil, found that casein was more protective against oxidation than whey proteins [32]. In another study examining the MDA content in baby biscuits prepared with and without milk, it was found that the MDA content in baby biscuits with added milk was decreased. This has been associated with the use of cow's milk, which has a higher casein content than whey protein [33]. Based on this information, the higher MDA levels observed in the biscuit group with higher protein content in the present study may suggest a potential association with protein-related factors; however, the contribution of other compositional or processing-related factors should also be considered, as the specific protein types of the products were not determined. Salt, heat, radiation, light, and food packaging are also effective in the formation of MDA. While this situation reduces the quality of the product, it also causes undesirable changes in the texture, taste, and color of the product that may affect human health [30, 34, 35].

As shown in Fig. 4, initial mean HMF levels tended to increase from high-fiber non-light to low-fiber non-light biscuits, whereas MDA levels were comparatively lower and showed less variation among biscuit groups. After in vitro gastrointestinal digestion, mean HMF concentrations increased in all groups, with higher values observed in low-fiber non-light biscuits. MDA levels also increased after digestion, although to a lesser extent than HMF. These results suggest that biscuit composition, including dietary fiber and carbohydrate content, may influence the levels and bioaccessibility of HMF and MDA under digestive conditions.

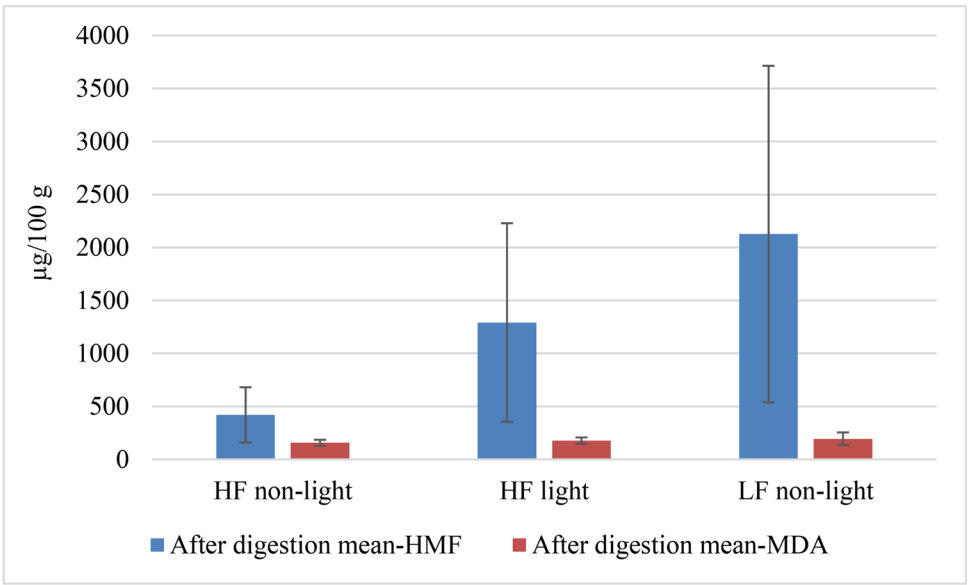
Bioaccessibility

Tables 2 and 3 show the results for HMF and MDA content in biscuits. The after-digestion HMF values in high fiber non-light, high fiber light, and low fiber non-light biscuits were determined by an in vitro digestion model including mouth, gastric, and small intestine (Table 2). Differences in after-digestion HMF value ranges were observed among biscuit groups. The bioaccessibility of HMF showed wide variability among biscuit groups, ranging from 56.4 to 309.7% in high-fiber non-light biscuits, 79.3-495.4% in high-fiber light biscuits, and 74.3-144.2% in low-fiber non-light biscuits. Overall, HMF levels increased after digestion in most products compared to initial values, with only a limited number of samples showing no increase (Table 2). The increase in HMF levels after digestion may be associated with the release of Amadori products bound to proteins during digestion and their subsequent transformation into HMF [36]. A study has also stated that the globular protein nature of milk and egg white proteins used in cake formulations can be associated with this transformation [37]. Since cakes and biscuits have similar formulations, this may partly contribute to the observed increase in HMF values in general in after-digestion products in our study. In addition, the Maillard reaction may be influenced by lipid oxidation processes, the acidic conditions of the gastric environment, and the further increase in environmental acidity by unsaturated fatty acids are other mechanisms associated with the increase in the amount of HMF [36]. It has been confirmed in the literature that sugar dehydration products such as 3-deoxyglucosone and 3,4-dideoxyglucosone accumulated in biscuits during baking transform into HMF under the acidic conditions of the gastric environment, causing a significant increase in the HMF content of biscuits at the end of this phase [38].

In our study, it was found that HMF content was generally lower in biscuits with high-fat content after digestion.



(a)



(b)

Fig. 4 Comparative analysis of mean hydroxymethylfurfural (HMF) and malondialdehyde (MDA) levels among different biscuit groups: (**a**) initial values and (**b**) after in vitro gastrointestinal digestion. Values are expressed as mean ± SD

In a study [36], it was found that in cakes formulated separately with hazelnut oil, sunflower oil, hazelnut oleogel, sunflower oleogel, and margarine, it was determined that the HMF concentration was lower in those with margarine both before and after digestion. Thus, since oils with different saturation levels were also used in biscuit formulations, it can be thought that this situation affected the HMF results in our study. Under high-temperature conditions, products with high carbohydrate and sugar content pose a significant risk in terms of HMF formation [38]. In our study, products with higher carbohydrate content were generally found to have higher HMF levels, while both products with high sugar content and products with relatively lower sugar content both had high HMF levels. This may be related to the type of carbohydrate, different carbohydrate compositions, the capacity of carbohydrate conversion to reducing sugars during processing, and the varying levels of reducing sugars [39].

The after-digestion MDA values of high-fiber non-light, high-fiber light, and low-fiber non-light biscuits are presented in Table 3. Differences in after-digestion MDA value ranges were observed among biscuit groups. The

Table 3 Amount and bioaccessibility of MDA in some creamless biscuits initially and after in vitro digestion

	Sample Name	Initial value (µg/100 g)	After Digestion (µg/100 g)	Bioaccessibility (%)
High fiber content creamless biscuits (non-light) (100 g)	1	40.2±2.0^{a}	113.2±5.3^{b}	281.6±13.1
	2	83.4±2.8^{a}	166.4±8.3^{b}	199.6±9.9
	3	70.7±2.6^{a}	158.6±7.1^{b}	224.4±10.1
	4	100.5±5.1^{a}	198.3±7.5^{b}	197.3±7.5
	5	77.9±3.3^{a}	170.7±5.5^{b}	219.0±7.0
	6	55.7±2.8^{a}	131.3±2.9^{b}	235.7±5.3
	7	63.1±2.9^{a}	135.6±4.6^{b}	215.0±7.2
	8	80.1±3.5^{a}	178.9±8.2^{b}	223.3±10.2
High fiber content creamless biscuits (light) (100 g)	9	91.2±4.6^{a}	198.1±14.0^{b}	217.2±15.4
	10	94.6±5.5^{a}	186.0±10.0^{b}	196.7±10.6
	11	91.5±6.5^{a}	175.9±4.6^{b}	192.3±5.0
	12	67.3±5.1^{a}	130.1±7.4^{b}	193.5±11.1
	13	99.6±6.4^{a}	225.7±12.2^{b}	226.6±12.3
	14	94.4±6.5^{a}	177.5±9.9^{b}	188.0±10.5
	15	48.9±3.1^{a}	189.3±10.0^{b}	387.2±20.5
	16	80.9±3.9^{a}	153.9±6.4^{b}	190.2±7.9
Low fiber content creamless biscuits (non-light) (100 g)	17	70.0±4.9^{a}	172.8±11.7^{b}	246.7±6.7
	18	63.0±5.1^{a}	144.2±5.5^{b}	229.1±8.7
	19	57.5±5.2^{a}	168.8±7.8^{b}	293.4±13.5
	20	92.5±6.3^{a}	161.5±8.9^{b}	174.6±9.6
	21	51.9±3.6^{a}	167.9±7.1^{b}	323.3±13.7
	22	67.6±3.7^{a}	159.3±5.8^{b}	235.5±8.6
	23	70.9±3.2^{a}	256.6±15.2^{b}	361.8±21.4
	24	106.9±7.2^{a}	300.1±12.5^{b}	280.6±11.6

Values are means ± range, $n = 3$. The different letters (a, b) in the same row indicate statistically significant differences between initial and after-digestion values for each biscuit sample (ANOVA, $p < 0.05$, Tukey's test)

bioaccessibility of MDA showed considerable variability across groups, ranging from 197.3 to 281.6% in high-fiber non-light biscuits, 188.0-387.2% in high-fiber light biscuits, and 174.6-361.8% in low-fiber non-light biscuits. Overall, MDA levels increased after digestion in all products compared to their initial values (Table 3). The observed increase in MDA levels after digestion may be associated with enhanced MDA reactivity under acidic conditions. This effect could be related to the low pH of gastric fluid, although the contribution of other factors, such as digestive enzyme activity or food matrix interactions, cannot be excluded [40]. The increase or decrease in MDA concentration after digestion may depend on various factors such as the type of fat present in the biscuits and the simultaneous presence of other components with pro-oxidant or antioxidant behavior, and the type of digestion model utilized [41]. In the literature, although the possible effect is limited between the fat content (EPA + DHA) of marine oils and the formation of MDA during in vitro digestion, it has been shown that naturally occurring components such as astaxanthin, polyphenols, tocopherols, and phospholipids (however, phospholipids may also have a pro-oxidative effect), depending on the type of oil, may have a protective effect against lipid oxidation [42]. In our study, it was found that MDA content was generally lower in biscuits with high-fat content after digestion. Our results differ from the results of studies on products with naturally low antioxidant content, such as meat products, and are closer to the results of products with relatively similar content, such as healthy diet bars [40, 43, 44]. In addition, the amount and type of protein in foods can significantly affect the level of oxidation of lipids during digestion [41]. In our study, it was found that MDA content was generally higher in biscuits with high protein content after digestion. During in vitro digestion, soy protein isolate inhibits the course of lipid oxidation, produces hydrolysis products with antioxidant properties that alter reaction pathways, and converts hydroperoxides to more stable hydroxides, processes that show that it slows down the oxidation of lipids under digesting circumstances [41]. In a study conducted by Carrillo et al. (2017), milk protein hydrolysates showed high inhibition of lipid peroxidation both in vivo and in vitro depending on the dose. It was determined that casein hydrolysates increased this effect even more with heat treatments [45]. Although many types of biscuits contain milk and soy products, the fact that high-protein biscuits in our study generally contain higher levels of MDA may suggest that a smaller portion of protein sources consist of these products. Therefore, increasing milk protein and soy protein isolates in biscuit types may be considered as a potential approach for reducing MDA formation.

The effect of antioxidants, fibers, sugar types, fat composition, protein sources, food additives and many other components used in various biscuit formulations on the formation and bioaccessibility of HMF and MDA needs to be further investigated.

Today, the consumption of processed foods in the daily diet has an important place in the fast pace of life due to reasons such as practicality, easy accessibility, long shelf life, flavor diversity, dietary product options, affordable prices, advertising, etc. However, processed foods may contain components that are harmful to health due to their formulation or processing techniques and processes. Knowing the toxic compounds, it contains, as well as the nutritional composition of the food consumed, makes a significant contribution to creating a healthy diet model that protects against diseases. Further studies are needed to understand the reasons for the formation of toxic compounds such as HMF and MDA in biscuits, which are important processed products. However, our study contains remarkable findings regarding both pre-digestion and post-digestion HMF and MDA contents of biscuits commonly sold in Türkiye.

Despite the valuable insights provided by the present study, several limitations should be acknowledged. First, the use of an in vitro gastrointestinal digestion model may not fully reflect the complexity of in vivo digestion and absorption processes. In addition, the specific types of dietary fiber, antioxidants, and lipid composition present in the biscuit formulations were not individually characterized, which may influence the formation and bioaccessibility of HMF and MDA. Future studies may address these limitations by incorporating in vivo models, evaluating the effects of different fiber types and concentrations, and investigating the role of antioxidants, protein sources, and fat composition on the formation and bioaccessibility of HMF and MDA.

Conclusion

Present study evaluated HMF and MDA levels in biscuits under digestive tract conditions. Initial HMF levels were lower in both light and non-light high-fiber biscuits compared to low-fiber biscuits. Fiber is essential in the food industry for enhancing food texture, viscosity, and gel formation, as well as for its health-promoting benefits. Additionally, high fiber content can enhance the quality and safety of food products. Despite their higher sugar content compared to most other biscuits, high fiber non-light biscuits had the lowest initial HMF levels. Similarly, MDA levels was found to be lower in high fiber biscuits. However, the highest MDA values were belong to the high fiber light-biscuits. The elevated levels of unsaturated fatty acids present in these products were likely responsible for this phenomenon. Briefly, the main findings of the present study revealed that HMF (except for 5 samples) and MDA levels in the products increased under in vitro gastrointestinal digestion conditions. Parameters such as product formulation, the presence or absence of antioxidants, and the pH level of the gastrointestinal digestive system may influence the formation of HMF and MDA. Moreover, high carbohydrate content increased HMF and MDA levels under digestive conditions. In contrast, MDA levels were observed to be lower in high-fat biscuits after digestion. This unexpected result can be attributed to the lower initial MDA level observed in high-fat biscuits. Also, bioaccessibility of HMF and MDA was lower in high-fat biscuits. Furthermore, bioaccessibility of MDA was observed to be lower in high-fiber biscuits.

Further investigation is required to elucidate the manner in which the diverse components present in biscuit formulations, including sugar, protein, antioxidants and fibers, influence the bioaccessibility of HMF and MDA. Such components may interact in disparate ways with HMF and MDA, potentially affecting their stability, release, and absorption within the gastrointestinal tract. A comprehensive investigation of these interactions would provide invaluable insights for the optimization of formulation strategies with the objective of enhancing the nutritional quality and safety of biscuit products.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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