Research article

5-hydroxymethylfurfural content in selected gluten- and gluten-free cereal food products

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Abstract: *5-Hydroxymethylfurfural (HMF) was determined with HPLC method in forty one food samples, including gluten-free, breakfast cereals and bakery products. The highest concentration of HMF was found in wheat bread with cranberries (210 mg kg^{-1}) and in breakfast cereals – honey wheat loops (85.099 mg kg^{-1}). In contrast, wholegrain oatmeal and gluten-free sponge cakes had the lowest HMF level of all tested samples, below the detection limit and 0.485 mg kg^{-1}, respectively. In most cases, lack of gluten coincided with the lowest HMF content readings (average 8.488 mg kg^{-1}). The impact of the type of sugar, especially glucose, on the HMF concentration in food is apparent. Sweetened breakfast cereals, with the average content of HMF at 25.55 mg kg^{-1}, took lead over dietary products (8.488 mg kg^{-1}) and bakery products (18.395 mg kg^{-1}), with the exception for wheat bread with cranberries. These cereals contained glucose or glucose-fructose syrup.*

Keywords: *hydroxymethylfurfural, gluten, gluten-free products, breakfast cereals, bakery products.*

Introduction

5-hydroxymethylfurfural (HMF) is a common component of heat-treated, dried or long stored food products. It is an intermediate formed in the Maillard reaction as a result of thermal dehydration of reducing sugars [1-2]. HMF is also formed during caramelisation, when carbohydrates degrade at high temperature [3].

As one of many compounds, HMF is responsible for the sensory properties of food, especially for the pleasant flavor. Although it is a relatively safe food component, there are reports regarding toxic, mutagenic and carcinogenic properties of 5-hydroxymethylfurfural, and – most notably – 5-sulphoxymethylfurfural (SMF), an allylic sulfuric acid ester metabolite formed from HMF [4-10]. In toxicological studies in mice and rats, an acute oral median lethal dose (LD50) was established at the level of 1910 mg kg^{-1} bw and 3100 mg kg^{-1} bw, respectively [10]. The value of no observed adverse effect level (NOAEL) is in the range of 80-100 mg kg^{-1} bw [4]. Therefore the acute toxicity of HMF is relatively low.

Considering the potential carcinogenic activity, there are several studies suggesting that HMF may act as initiator and promoter of colon cancer in rats [11-12]. It was shown that HMF induces an elevated number of preneoplastic aberrant crypt foci (ACF) by the induction of gene mutations in the colon mucosa. Although HMF itself is not mutagenic, its metabolite SMF has mutagenic potential [4].

It is also important to note that the data on the toxicological and carcinogenic potential of HMF is very limited. The conclusions from studies on mechanism of its toxicity are insufficient and controversial. This makes it impossible to establish a tolerable daily intake (TDI) of hydroxymethylfurfural [4-5, 8].

Hydroxymethylfurfural is present in many foodstuffs. The content of HMF in the various products vary within a wide range and depends on the food type as well as the type of processing. The main source of HMF in human nutrition are cereal products (bread, breakfast cereals, cookies), coffee, dried fruits, beverages – including alcoholic drinks, chocolates, milk and honey [4, 13-18]. The highest concentrations of HMF are reported for dried fruits, especially plums with up to 2200 mg kg^{-1} [15]. According to data provided by the Spanish researchers, bread and coffee are the most important source of dietary HMF [17]. Although the concentration of HMF in bakery products and in coffee is relatively low, in the range of 4.1 to 151.2 mg kg^{-1} [16], and of 12 to 300 mg kg^{-1} [13], respectively, its makes up nearly 85% of the total HMF daily exposure. Cereals and cereal products, especially bread, are also identified as the main source of HMF in the diet by German and Norwegian researchers [4, 14].

The content of HMF in fresh honey is low and generally does not exceed 15 mg kg^{-1} [19]. However, technological processing it is subjected to, such as thermal treatment, as well as long-term storage, cause significant increase in HMF level. For this reason, the level of HMF in honey is a good indicator of its freshness and quality. Having that in mind, Codex Alimentarius Commission (ALINORM 01/25, 2001) and the European Union (DIRECTIVE 2001/110/EC) established a maximum HMF level in honey of 40 mg kg^{-1} (80 mg kg^{-1} in tropical honey).

HMF content in breakfast cereals may vary largely, depending on the type of grain and fruit or/and flavor additives (e.g. dried fruits, honey or cocoa). The average content is 7 to 114 mg kg^{-1} for cornflakes, 6 to 132 mg kg^{-1} for wheat-based cereals, and 6 to 240 mg kg^{-1} for cereals made from a mixture of grains [4, 17, 20].

Most sources report the value of HMF daily intake in the range from 4 to 30 mg per person. The exception to that being beverages made from dried plums, which can deliver even 350 mg of HMF per day [4]. Still, to this day hydroxymethylfurfural as a dietary compound have been poorly explored.

In connection with a deficit of literature data on the HMF content in dietary food products, especially gluten-free, it seems interesting to investigate whether there is a relationship between the presence of gluten and the level of HMF in selected cereal products.

Therefore, in this paper, the HMF content was determined in different types of breakfast cereals, cookies and muesli as well as in several types of commercially available bakery products. Moreover, the influence of flavour and taste ingredients (honey, cocoa, dry fruits) as well as the type of cereals (gluten or gluten-free) on the HMF content was examined in the tested products.

Experimental

Materials

All chemicals used were of analytical grade and were from Merck (Darmstadt, Germany).

Samples

Twenty-one commercial packaged breakfast cereals products (cornflakes, multigrain cereals, wholegrain oatmeal, with and without food additives), eight gluten-free or sugar-free dietary products (cookies, biscuits, sponge cakes and pasta) and twelve bakery products were collected from supermarkets.

Preparation of samples for analysis was performed according to the procedure given by Rufián-Henares *et al.* [20]. Ground sample (500 mg) was suspended in 5 ml of deionised water in a 10 ml centrifuge tube. The tube was shaken vigorously for 1 min and clarified with 0.25 ml each of potassium ferrocyanide (15% w/v) and zinc acetate (30% w/v) solutions. The resulting mixture was centrifuged at 4500 g for 10 min at 4°C. The supernatant was collected in a 10 ml volumetric flask and two further extractions were performed using 2 ml of deionised water. The supernatants were mixed and the volume was made up to 10 ml with deionised water. Analysis for HMF was made by HPLC on the filtered (0.45 mm) solution.

Methods

Analysis of HMF

The HPLC system used (Dionex) consisted of an P580 system controller pump, an degassing device, an Gina 50 autosampler, and an UVD170S UV detector. Data acquisition was accomplished by a Chromeleon 6.0 system. The separation was performed on a Hypersil BDS C18 column 4.6-250 mm, 5 µm particle size, with a matching guard cartridge.

Chromatographic Conditions: analysis was carried out isocratically at 40°C (±1) using as the mobile phase a mixture of methanol-water (15:85) (v/v) at a flow rate of 1 mL/min. The variable-wavelength detector was set as 283 nm. The total run time required was 15 min.

Since the concentration of HMF in analysed samples of food was very high, it was necessary to dilute the first extract ten times with deionized water before

HPLC procedure was carried out. This procedure was included in all further calculations.

Statistical analysis

Statistical significance of data was tested by one-way analysis of variance (ANOVA). Analyses were performed using Microsoft Excel Analysis ToolPack.

Numeric data are expressed as means ± SD. The statistical significance of differences in individual mean values was calculated using the unpaired, two-tailed Student's t-test with significance levels $p < 0.05$. Homogenity of variance was tested with the F-test (ANOVA, confidence interval at 95%).

All experiments were performed at least in three independent repetitions. Microsoft Excel 2007 was applied for statistical parameters.

Results and Discussion

The impact of gluten presence

Dietary products, especially gluten-free products have not been studied yet in terms of hydroxymethylfurfural content. Considering the HMF levels obtained in a group of dietary products, the lowest values were obtained for gluten-free products (mean 3.153 mg kg^{-1}), except for gluten-free low-protein sponge cakes. HMF content ranged from 0.485 to 11.929 mg kg^{-1} for gluten-free sponge cakes and gluten-free biscuits with raisins, respectively (Table 1). If we only consider gluten-free products with sucrose as the sweetening agent, or without it (Table 1, samples no. 1, 2 and 6), the average content of HMF was only 0.577 mg kg^{-1}. Significantly higher concentrations of HMF were determined in the gluten-free butter cookies, and in gluten-free biscuits with raisins, 2.106 and 11.929 mg kg^{-1} respectively.

Table 1. HMF content of dietary food products

No.	Product	HMF (mg kg^{-1})
1.	gluten-free sponge cakes	0.485 ± 0.017
2.	gluten-free sugar-free sponge cakes	0.500 ± 0.015
3.	gluten-free butter cookies	2.106 ± 0.056
4.	gluten-free biscuits with raisins	11.929 ± 1.003
5.	gluten-free low-protein sponge cakes	39.380 ± 2.311
6.	gluten-free pasta	0.746 ± 0.017
7.	sugar-free spelt cookies	8.678 ± 0.762
8.	sugar-free coconut cookies	4.081 ± 0.876

It is difficult to compare these results to other sources, because so far no studies have been published on the HMF content in gluten-free dietary products. Nevertheless, a higher HMF content in the two latter products could be explained by analyzing their composition. The sweetening agent in the gluten-free butter cookies is a glucose-fructose syrup, a source of glucose and fructose, what is the likely cause of the increased amount of HMF in the product. That is consistent with previous findings about the effect of glucose on the formation of HMF in final product. It was found that during baking process, glucose contributes to HMF formation to a much greater extent than sucrose [21-22]. The high content of HMF in gluten-free biscuits with raisins is primarily due to the participation of the dried fruit, what is thoroughly discussed in further part of present paper.

The highest content of HMF in the dietary products group was found for gluten-free low-protein sponge cakes and was 39.380 mg kg^{-1}. It had the highest calorific value in this group due to the high contribution of both vegetable fat and sugars (sucrose and glucose). A significant amount of glucose, the main precursor of HMF, implies large level of HMF in this product.

On the other hand, gluten-containing dietary products (Table 1, samples no. 7 and 8) were characterized by diverse HMF level, but in most significantly higher than the mean HMF content of the gluten-free products. For example, the HMF content of sugar-free coconut cookies was 4.081 mg kg^{-1}, while in sugar-free spelt cookies it was 8.678 mg kg^{-1}, both based on wheat. The sweetener in the coconut cookies is a maple syrup, a source of predominantly sucrose but also a small amount of glucose and fructose, and is responsible for higher level of HMF in the product. This is in conformity with the above-described effect of glucose on the content of HMF in confectionery [21-22]. While the spelt cookies, even though they do not contain any sweetener are characterized by a relatively high level of HMF. High levels of HMF in this case reflects to the way of dough production, that was fermented before baking. This issue will be discussed in further section of this paper.

Table 2. HMF content of breakfast cereals

No.	Product	HMF (mg kg^{-1})
1.	cornflakes (cheap brand)	14.28 ± 1.03
2.	cornflakes (leading brand)	20.06 ± 1.56
3.	cornflakes with honey and peanuts (cheap brand 1)	11.66 ± 1.66
4.	cornflakes with honey and peanuts (cheap brand 2)	10.08 ± 0.99
5.	cornflakes with honey and peanuts (leading brand)	8.25 ± 1.77
6.	honey wheat puffs	11.66 ± 2.31
7.	honey wheat loops	85.10 ± 3.34
8.	multigrain rings with honey	62.73 ± 2.67
9.	cocoa cereal balls (cheap brand 1)	15.07 ± 1.89
10.	cocoa cereal balls(cheap brand 2)	12.72 ± 0.88
11.	cereals with white and dark chocolate	49.70 ± 1.77
12.	cereals with dark chocolate	66.94 ± 2.33
13.	cereals with white chocolate	16.93 ± 0.99
14.	fitness flakes with dried fruits	6.78 ± 0.89
15.	muesli five grains with raisins	4.79 ± 1.22
16.	crunchy with fruits	6.06 ± 0.89
17.	rice-wheat flakes with fruits	11.69 ± 1.01
18.	oatmeal with red fruits	47.62 ± 2.21
19.	fitness flakes without additives	6.15 ± 0.33
20.	fitness flakes with yoghurt	21.99 ± 0.99
21.	wholegrain oatmeal	<*

* below the detection limit

There have been also analyzed twenty-one commercial breakfast cereals samples, seven of which does not contain gluten (Table 2, samples no. 1-5, 18 and 21), as declared in the product composition. In gluten-free breakfast cereals tested in this survey the mean content of HMF was 15.992 mg kg^{-1}, and ranged from below the detection limit (wholegrain oatmeal) to 47.619 mg kg^{-1} (oatmeal with red fruits). A wide range of results is probably due to the kind and amount of used sweetener and additives, primarily dried fruits. The values obtained by other researchers do not differ significantly from received by us. Teixidó *et al.* [18] presents a very similar HMF values for breakfast cereals based on maize and rice grains, mean 28.55 mg kg^{-1}. Whereas, Rufián-Henares *et al.*[20] found a mean content of HMF in maize and rice based breakfast cereals (42.87 and 32.14 mg kg^{-1}, respectively).

In summary, the presence of gluten in investigated samples, both in dietary products and in breakfast cereals, have some influence on higher HMF content. The average level of HMF in all investigated groups of gluten-free food was lower than in those with gluten. The mean HMF content in gluten-free dietary products group was 3.153 mg kg^{-1}, while for gluten-content dietary products it was 6.379 mg kg^{-1}. A similar trend was obtained for the analyzed breakfast cereals, for gluten-free samples the mean HMF level was 15.992 mg kg^{-1}, and 25.394 mg kg^{-1} for those with gluten.

The impact of presence and type of sugars

The type and amount of sweetener used in food products seems to be essential for final hydroxymethylfurfural level. This aspect has been widely studied on the example of various kinds of bakery and confectionery products [21-23]. All of these studies show strong dependence of the type and amount of sugars on hydroxymethylfurfural level in studied foodstuffs. Gökmen *et al.* [21] revealed linear relationship between the amount of sugar in the recipe and the amount of HMF formed in cookies upon baking. However the effect of glucose on HMF formation in cookies was much greater than the effect of sucrose. Similar conclusions have drawn Zhang *et al.* [22] on the basis of research on effects of sugars in batter formula on the hydroxymethylfurfural and furfural (F) formation in sponge cakes. They observed that the type of sugar added in addition to the sucrose base exert strong influence on HMF and F formation in sponge cakes. Moreover sucrose, lactose and maltose yielded less HMF that did glucose and fructose. Zhang's results were confirmed in Petisca *et al.* [23] survey, where biscuits presented the highest HMF levels, compared to studied bread and cake samples, due to their highest carbohydrate, mostly glucose-fructose syrup amount, as well as the lowest moisture content.

These findings were confirmed in our study. Both dietary products and breakfast cereals sweetened with glucose and/or glucose-fructose syrup had higher HMF content vs. products sweetened with sucrose or sugarless products. For example, sucrose sweetened gluten-free sponge cakes, containing up to 66.5 g of carbohydrates per 100 g, had the lowest content of HMF (0.485 mg kg^{-1}) in a group of dietary products. While gluten-free low-protein sponge cakes, sweetened with glucose and sucrose, had more than eighty times higher content of HMF, i.e. 39.380 mg kg^{-1} (Table 1).

A similar trend was observed in the group of breakfast cereals, although not as strong as in the case of dietary products. The mean content of HMF in analyzed breakfast cereals with sugars was 25.55 mg kg^{-1}, from 6.064 mg kg^{-1} to 85.099 mg kg^{-1}, for crunchy with fruits and honey wheat loops, respectively (Table 2). Both samples contained sugar, and crunchy with fruits had also glucose-fructose syrup and caramelized sugar, whereas honey wheat loops had also glucose syrup, honey and molasses. The vast majority of the analyzed breakfast cereals contained in its composition sucrose, glucose syrup or glucose-fructose, and some of them also honey, molasses and caramelized sugar.

Considerably lower content of HMF was observed for two sugar-free breakfast cereals samples tested in our research. HMF level in these samples ranged from below the detection limit (wholegrain oatmeal) to 4.798 mg kg^{-1} (muesli five grains with raisins).

The impact of flavour and taste ingerdients

Further grouping was made to determine the influence of different ingredients added to tested food products such as dried fruit, cacao powder or chocolate, and honey.

According to the literature, the HMF content in dried fruits is relatively high. Murkovic & Pichler [15] found the highest concentration of HMF in dried plums (up to 2200 mg kg^{-1}), and slightly lower level in dried dates (1000 mg kg^{-1}). Other fruits, like apricots, pears, peaches, figs, apples and pineapples had much lower concentration of HMF.

Depending on the amount and type of dried fruits in the tested food products different HMF levels can be expected. In the group of dietary products dried fruits were only in gluten-free biscuits with raisins, containing HMF of 11.929 mg kg^{-1}. Raisins, like other dried fruits, are described as a relatively rich source of HMF, mean 33.50 mg kg^{-1} [24]. To a lesser extent, the high level of HMF in this product may be affected by the presence of sucrose, both inside and on the surface of the biscuit.

Among the analyzed breakfast cereals with dried fruits, those with raisins were characterized by similar level of HMF, i.e. 11.697 mg kg^{-1} for rice-wheat flakes with fruits, and 6.783 mg kg^{-1} for fitness flakes with dried fruits. Striking result is the low concentration of HMF obtained for crunchy with fruits, such as raisins and plums (6.064 mg kg^{-1}). While the highest concentration of HMF was obtained for oatmeal with red fruits (apples, strawberries and red currants), 47.619 mg kg^{-1}. The average content of HMF in five tested breakfast cereals samples with fruits (Table 2, samples no. 14-18) was 15.392 mg kg^{-1}. This value is relatively low in relation to the mean content of HMF in breakfast cereal group (23.347 mg kg^{-1}), which indicates a much greater impact of presence and type of sugars, in particular glucose, and glucose-fructose syrup on the HMF level than the presence of dried fruit.

However, we noticed a significant effect of the addition of dried fruit on HMF concentration in breads. We analyzed nine bakery products, it is bread made from wheat, rye, mixed with seeds and dried fruits (Table 3). The highest concentration of HMF was in bread with cranberries (210.00 mg kg^{-1}) and in wholemeal bread with raisins (40.27 mg kg^{-1}). On the other hand, the lowest level of HMF was in whole-wheat loaf B, only 2.223 mg kg^{-1}. The average content of HMF in bread without dried fruit was 13,771 mg kg^{-1}. Our results are consistent with those obtained by Ramírez-Jiménez *et al.* [25]. They reported the HMF concentration in white bread with fruits as 51.3 mg kg^{-1}. In turn, Murkovic & Pichler [15] determined the HMF level in bread with dried fruits as 450 mg kg^{-1}.

Table 3. HMF content of bakery products

No.	Product	HMF (mg kg^{-1})
1.	rye bread A	36.72 ± 1.72
2.	rye bread B	17.05 ± 0.79
3.	wheat-rye bread A	12.46 ± 1.77
4.	wheat-rye bread B	28.05 ± 1.89
5.	wholemeal bread	28.69 ± 1.09
6.	wholemeal bread with seeds	7.56 ± 1.06
7.	wholemeal bread with raisins	40.27 ± 0.97
8.	wheat bread with cranberries	210.00 ± 4.46
9.	whole-wheat loaf A	16.49 ± 0.88
10.	whole-wheat loaf B	2.23 ± 0.11
11.	wheat baguette	9.88 ± 0.32
12.	ciabatta	2.94 ± 0.24

Honey in cereal products acts both as a sweetener and flavoring agent. Its presence in analyzed breakfast cereals (Table 2, samples no. 3-8) significantly increased concentration of HMF (mean 31.581 mg kg^{-1}) in comparison to sweet flakes without honey (mean 22.770 mg kg^{-1}). Our results confirm the results obtained by other researchers. For example Rufián-Henares *et al.* [20] obtained the mean HMF level equal 43.44 and 34.24 mg kg^{-1}, respectively for breakfast cereals with and without honey. A similar level of HMF in breakfast cereals with honey recorded Teixido *et al.*[18], 41 mg kg^{-1}.

Other grouping was made to determine the influence of cocoa powder added during breakfast cereal manufacture on HMF level. The average content of HMF in tested cereals with cocoa powder was 36.107 mg kg^{-1} (Table 2, samples no. 9-12), and was higher than its concentration in cereals without this additive (mean 22.740 mg kg^{-1}). This is contradictory to the results obtained by Rufián-Henares *et al.*[20]. Authors found no differences in HMF concentration when cocoa powder was added or not to cereals.

The impact of dough fermentation process

Studies indicate that fermentation process of dough significantly increases the HMF level in the final product, and it can varies considerably depending on the duration of fermentation for some ingredients and baking conditions [25-26]. In particular, rye flour produces higher amount of HMF, probably because of the higher free amino acids or proteins content [27]. This phenomenon is particularly evident in the case of bread and other bakery products.

These observations were confirmed in our study. We determined the concentration of HMF in twelve bakery products derived from rye, wheat and/or wholemeal flours (Table 3). Results for two types of analyzed bread (wholemeal bread with raisins and bread with cranberries) were discussed in the section on the impact of food additives on HMF content, and were excluded from this group. The average content of HMF in fermented breads (Table 3, samples no. 1-5) was 24.977 mg kg^{-1}, while in rye bread it was higher (mean 26.885 mg kg^{-1}) than in wheat-rye bread (mean 23.069 mg kg^{-1}). In addition, we determined the concentration of hydroxymethylfurfural in bread flours, wheat, whole-wheat, and rye flours. All of these samples showed the HMF level below the detection limit (data not shown).

Similar levels of HMF in wheat-rye bread received Serpen *et al.* [26] (10.6 mg kg^{-1}). Whereas the HMF content in wheat bread was much lower, average 7.8196 mg kg^{-1}, similarly as in Ramírez-Jiménez *et al.* [25] survey, where they obtained 7.4 mg kg^{-1} for whole white bread.

Our results confirm previous observations indicating the importance of the type of flour used in the production of bread, as well as the process of dough fermentation. The dough prepared with wheat flour or wheat-rye flour is subjected to a different type of fermentation, which requires a longer time than yeast fermentation of wheat dough. This entails a greater share of reducing sugars in the dough, resulting in a much higher concentration of HMF in bread.

Thus, the dough fermentation process, especially its length has a significant impact on the level of HMF in the finished product.

Summary

In conclusion, on the basis of the results obtained from testing forty-one samples of food products, it is the type and quantity of sugars, especially glucose, that have the most impact on the HMF level. This is particularly evident in the case of dietary products and breakfast cereals analyzed in this survey. So does the type of grain used as raw material for food manufacturing. The difference between HMF content and the type of food product is statistically significant ($p < 0.05$) in case of all three tested group of food. In addition, our research confirms the findings of other researchers, indicating the effect of the addition of dried fruits as well as the fermentation process, on HMF content in cereal products, especially bread. The influence of dough fermentation process mostly applies to bakery products made from rye flour.

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