Hydroxymethylfurfural in commercial biscuits marketed in Spain

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Summary

Hydroxymethylfurfural (HMF) contents were evaluated in sixty-two commercial digestive and semi-sweet biscuits marketed in Spain. Values ranged from 3.1 $mg\cdot kg^{-1}$ to 182.5 $mg\cdot kg^{-1}$, with an average of 14.4 $mg\cdot kg^{-1}$ with a huge variability. Influence of dough composition on HMF level was also investigated. Replacing reducing saccharides by saccharide alcohols such as maltitol or lactitol will significantly reduce the formation of HMF during baking. Distribution of HMF in different zones through the biscuit was analysed and, as expected, HMF was heterogenously distributed, with the highest percentage of the compound located in the upper and border sides of the biscuits. This study establishes a dietary exposure of the Spanish population to HMF from biscuits as 2.3 μg per kg of body weight per day.

Keywords

hydroxymethylfurfural; biscuits; dietary intake; Maillard reaction

In the biscuit formulation, the main ingredients are cereal flour, saccharides and fats, later the dough is conventionally baked at high temperature and short times (up to 200 °C for less than 20 min) in order to reduce the water content (< 10%) and to promote a brown surface [1]. Baking is a complex process inducing physical, chemical and biochemical changes in the cereal matrix such as volume expansion, evaporation of water and formation of a porous structure, denaturation of proteins, starch gelatinization, crust formation and development of a desirable taste and pleasant flavours and browning [2]. The chemical reactions involved in the generation of colour and flavour are essentially the Maillard and caramelization reactions, which both depend on the type of substrate, temperature, water activity and pH [3].

The Maillard reaction (MR) occurs between the free amino group of lysine and/or other amino acids and the carbonyl groups of reducing saccharides, such as glucose or maltose [4]. The reaction is favoured in systems with intermediate moisture contents, temperatures over 50 °C and pH 4–7, whereas caramelization depends on direct degradation of saccharides and requires more vigorous conditions, such as temperatures higher than 120 °C, pH lower than 3 or higher than 9, and low water activity [5]. In addition, the heating conditions used during baking favour the hydrolysis of starch and non-reducing saccharides, forming reducing saccharides that can participate in both reactions. Thus, MR and caramelization may occur simultaneously.

The loss of available lysine and accumulation of undesirable compounds generated during the advanced stages of MR, such as furanic compounds, are commonly measured to evaluate the severity of the heat treatment applied and the effect of storage [6]. Upon heating at high temperature, furanic compounds are generated by two possible pathways: (i) the caramelization, where the reducing saccharides including maltose and maltotriose [5] directly undergo 1–2 enolization, dehydration and cyclization reactions; and (ii) the Maillard reaction, where the Amadori product, formed by the reaction with the amino group of free amino acids or proteins, is submitted to enolization and subsequent dehydration of the saccharide moiety while releasing the amino acid intact [3]. 5-Hydroxymethyl-2-furfuraldehyde (HMF) is formed by both reactions and is considered as the essential decomposition product of hexoses. Levels of HMF

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have been reported in cereal products such as baby cereals [7] and breakfast cereals [8], pasta [9] and bakery products [10]. Recently, some concern arises from the toxicological relevance of HMF since in vitro studies on genotoxicity and mutagenicity have given controversial results [11, 12].

In this study, the presence of HMF in commercial biscuits, designated as digestive and semi-sweet, was investigated with the aim of screening its level in this food sector. The effect of different ingredients in the dough formulation on HMF formation was examined based on the labelling information, as well as HMF distribution in different zones through the biscuit was determined.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade and were from Merck (Darmstadt, Germany), unless mentioned otherwise (see Rufián-Henares et al. [13] for further details).

Samples

Sixty-two commercial biscuits marketed in Spain produced by 15 different producers were purchased from supermarkets. Products containing chocolate, dried fruits or cream were omitted from the sampling. The samples were randomly named using a number. The total content of each package was powdered in a grinder, homogenized and stored in polyethylene containers under vacuum at 4 °C until analysed.

Analysis of HMF

HMF determination was based on the method of Rufián-Henares et al. [13]. The HPLC system consisted of a MD-420 pump, a MD-465 autosampler, a MD-432 ultraviolet-visible detector and a DT-450/MT v. 3.90 computing integrator connected to a PC, all from Kronton Instruments (Milan, Italy). HMF was quantified using the external standard method within the range of 1–200 $\mu mol \cdot l^{-1}$ and 1–20 $\mu mol \cdot l^{-1}$, respectively. Limit of quantitation was set as 0.05 $mg \cdot kg^{-1}$. Each sample was analysed in duplicate and the mean of two measurements was reported.

Measurement of colour

Colour measurements were performed on the ground and homogenized samples using a Hunter Lab D25-9 optical sensor (Hunter Associated Laboratory, Reston, Virginia, USA) according to the CIE Lab scale [14]. The system provides values of three colour components: L^*, a^* and b^*. Samples were placed into a glass Petri dish of a diameter of 5 cm. The sample was illuminated with D65-artificial daylight (standard angle, 10°) according to conditions provided by the manufacturer. The ΔE was calculated from the equation:

$$\Delta E = (L^{*2} + a^{*2} + b^{*2})^{1/2} \quad (1)$$

Statistical analysis

Statistical significance of data was tested by one-way analysis of variance (ANOVA), followed by the Duncan test to compare means that showed significant variation ($P < 0.05$), and Chi-square test for normality; these were performed using Statgraphics Plus, version 5.1, 2001 (Statistical Graphics Corp., Rockville, Maryland, USA).

RESULTS AND DISCUSSION

Sample description

Usual commercial dough formulation contains flour (mainly wheat), saccharide, fat and water, together with a number of common additional ingredients in a minor proportion, like salt or leaving agents. The survey was focused on digestives and semi-sweet biscuits without fillings with chocolate or cream. Tab. 1 summarizes the main information

Tab. 1. Summary of the energy, protein, carbohydrate, fat and fibre contents in 100 g of commercial biscuits as declared by the manufacturers.

	Energy [kJ]	Protein [%]	Carbohydrate [%]	Fat [%]	Fibre [%]
mean	1889.1	7.8	67.9	16.5	5.7
standard deviation	117.6	1.5	7.1	4.0	3.8
relative standard deviation	26.0	18.9	10.5	24.4	67.4
minimum	1548.8	5.3	45.5	7.0	1.0
quartile1	1827.2	7.0	63.6	15.3	3.0
median	1904.6	7.5	68.0	17.8	4.6
quartile2	1983.7	8.1	72.6	19.0	7.0
maximum	2051.1	12.0	81.0	21.8	16.5

Tab. 2. List of different sources of saccharides, cereal flour and other ingredients commonly present in the dough formulation for biscuits.

Carbohydrate	Cereal	Other
saccharose	wheat	dried whey
glucose	oat	dried skimmed milk
fructose	rice	dried eggs
inverted saccharide syrup	malt	almonds
saccharide cane	barley	casein
brown saccharide cane	soy	Inulin
(honey)		L-carnitine
wheat starch		soy
potato starch		PUFA
maize starch		sesame
caramel		
lactitol		
maltitol		
maltitol syrup		
oligofructose		

related to macronutrients and energy intake as obtained from manufacturers. Levels of major constituents were similar in all the samples except for the fibre content whereas the interquartile range was increased by two-fold. Based on this, it was reasonable to divide the samples for further studies in two groups regarding the fibre contents.

Tab. 2 summarizes a list of potential ingredients used in the formulation as described by manufacturers. It is noteworthy that some of the ingredients may already contain significant levels of HMF, like honey or syrups. Wheat flour or wheat flour in combination with wheat bran is mainly used as a unique cereal for formulation in nearly 62% of the samples. The rest contain wheat flour added with other cereals (oat, rice, malt, barley or soya). Dried whey and dried skimmed milk is widely used in the formulation, while inulin, polyunsaturated fatty acids and L-carnitine are mainly restricted to products with a specific dietetic orientation. Commercial biscuits are usually baked at temperatures around 200 °C for 20 min but the final water content and browning are commonly used to determine the end of the baking process.

HMF content

A detailed study of HMF distribution in commercial biscuits was carried out, and the results

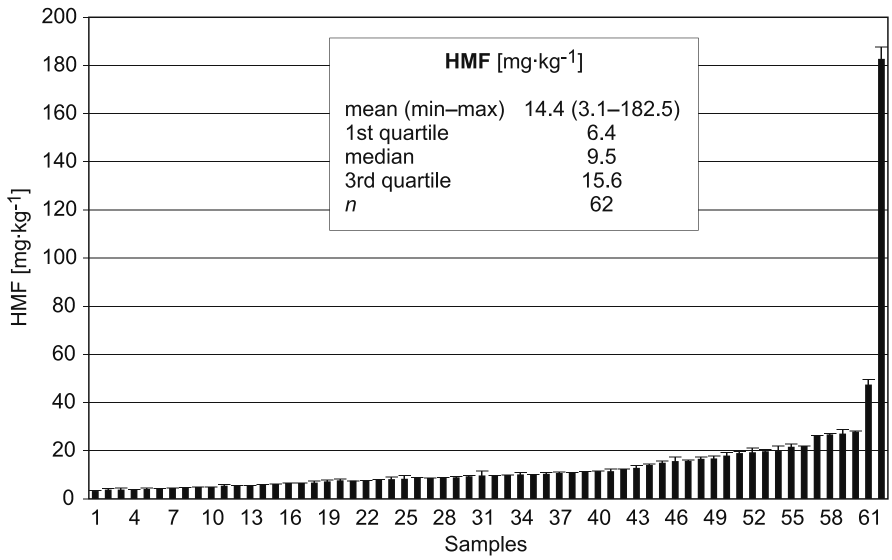

Fig. 1. HMF contents and distribution in commercial biscuits marketed in Spain (mg·kg-1).

were graphically presented as a box-and-whisker plot and a bar graph since these graphical presentations use a non-parametric test (Fig. 1). HMF contents ranged from 3.1 $mg\cdot kg^{-1}$ to 182.5 $mg\cdot kg^{-1}$ with a mean value of 14.4 $mg\cdot kg^{-1}$ and a median of 9.5 $mg\cdot kg^{-1}$. Three outliers where detected from the distribution (43.8, 47.3 and 182.5 $mg\cdot kg^{-1}$) corresponding to traditional digestive biscuits, which contain glucose syrup and wheat flour. Skipping samples containing syrup, the high variability in HMF among samples could not be directly attributed to any specific ingredient and must be related to both the most severe heat treatment and/or a variation in the dough formulation not declared by manufactures, which might have affected some reactants. Results for HMF contents are in the same order of magnitude as reported by AIT-AMEUR et al. [1] for biscuits marketed in France, ranging from 0.5 $mg\cdot kg^{-1}$ to 78.6 $mg\cdot kg^{-1}$, with a mean value of 24.1 $mg\cdot kg^{-1}$ and a median of 19.2 $mg\cdot kg^{-1}$. RAMÍREZ-JIMÉNEZ et al. [10] reported HMF contents from 4.1 $mg\cdot kg^{-1}$ to 151.2 $mg\cdot kg^{-1}$ for selected bakery speciality products. GÖKMEN and SENYUVA [15] described slightly lower values of these products in a heterogeneous group of cereal-based foods (breads and biscuits), ranging from 0.2 $mg\cdot kg^{-1}$ to 57.2 $mg\cdot kg^{-1}$ (mean value of 5.3 $mg\cdot kg^{-1}$).

HMF content in food is currently under evaluation due to its possible toxicological relevance for human health [11, 16]. The carcinogenic potential of HMF is a consequence of its metabolic activation by sulfotransferases, which convert it to 5-sulfoxymethyl-2-furfural (SMF). This reaction may take place in the liver. The exceptionally high human exposure, ubiquity of HMF in the diet and genotoxicity results in vivo, prompted the investigation of the dietary exposure to HMF in order to update its toxicological relevance in foods. In the present study, HMF dietary exposure of the Spanish consumers from biscuits was estimated as 2.3 μg per kg of body weight per day, for a mean body weight of 70 kg and the intake of these food commodities supplied by the Spanish Ministry of Agriculture, Fish and Alimentation [17].

A previous study of our research group evaluated the HMF content in a diet usually consumed by the adolescent population in 3.87 $mg\cdot kg^{-1}$ dry matter [18], what supposed an HMF daily intake of 2.36 $mg\cdot d^{-1}$, or 33.76 $\mu g\cdot kg^{-1}d^{-1}$. Taking into account this whole daily intake of HMF, the contribution of biscuits can be estimated by 6.8%. This not very high percentage is in agreement with the fact that biscuits represent only 6% of the total ingest of cereal-derived products consumed by this population in the cited study, whereas bakery and breakfast cereals were the major contributors [19].

Besides these data of HMF intake, bioavailability studies are needed to evaluate the real input of HMF to the organism.

Relationship with formulation

As previously described, biscuits are a group of heterogeneous food commodities because they are composed of a great variety of ingredients. Then, in order to find out the possible influence of different amounts and varieties of ingredients on the HMF content, biscuits were grouped according to different variables declared by manufacturers. Outliers were omitted from the analysis to avoid any bias and to improve the homoscedasticity of the distribution and subsequently the strength of the analysis. Variables selected at two levels were dietary fibre content (higher / lower than 5%), type of flour (wheat / multicereal), use of syrup (present / absent) and use of polyalcohols (present / absent). Results recently published in the literature have shown that high fructose [1] or ammonium bicarbonate levels [20] promote the formation of HMF during baking. Replacing ammonium bicarbonate with sodium bicarbonate maintained pH of biscuits between 9.0 and 10.0 during baking, which limited the decomposition of saccharose and the subsequent formation of HMF. However, levels of fructose or type of leavening agent used was not supplied by manufacturers for all the samples.

The content of the dietary fibre or the type of flour were not found to have a significant influence on the HMF content. Addition of wheat bran did not contribute to the generation of HMF, which is in line with previous results for breakfast cereals [8]. However, HMF was significantly lower ($P = 0.04$) in commercial samples with added polyols (6.8 $mg\cdot kg^{-1}$; $n = 6$) as compared with samples without polyols (15.2 $mg\cdot kg^{-1}$; $n = 53$) in the dough formulation. Replacement of reducing saccharides by polyols such as maltitol or lactitol in the dough formulation will give rise to a decrease on the extent of the browning reactions. At these conditions, formation of HMF is limited during the baking process. Therefore, high temperatures employed during the baking process can lead to the predominance of caramelization over MR, which is reinforced by the presence of an important amount of reducing saccharides as substrates for the caramelization reaction. Similar behaviour has been described for acrylamide, a newly established processing contaminant, in biscuits [21].

HMF content was significantly higher ($P = 0.03$) in samples with addition of syrup (17.8 $mg\cdot kg^{-1}$, $n = 35$) than without addition of syrup (9.0 $mg\cdot kg^{-1}$, $n = 24$). These findings will limit the application of HMF for assessing the baking condition in commercial biscuits, but HMF is still useful for internal quality control of the process for same formulation. Effect of the addition of syrup or replacement of saccharides by polyols was even more evident if the study was restricted to the group of biscuits with only wheat as a flour source.

Mapping of the HMF distribution in biscuits

Formation of HMF is related not only to the formulation but also to the baking conditions and technology applied. Technical information on baking conditions was not supplied by manufacturers. The distribution of HMF in different zones of the biscuit was studied. This is characteristic to the baking approach used. Six different samples with a regular shape (circular or rectangular) were included in this study. A portion of the upper (US) and the lower side (LS) was obtained by scraping off the biscuit from several units and pooled separately. In a similar way, from some other units, a fraction of sample was obtained from the middle area of the biscuit (MS) and the border side (BS) as well. In a preliminary step, colour (ΔE) distribution in each portion, as compared with the total biscuit, was studied to identify lack of uniformity in the heat load (data not shown). US was found to be significantly browned as compared to LS for three of the samples indicating that heat load was not uniformly distributed, whereas MS and BS did not show significant differences.

HMF content was analysed in US, LS, MS and BS portions to map the contribution of each fraction to that of the whole biscuit. Results are summarized in Fig. 2. As expected, HMF content was increasing from LS to US and from MS to BS. Highest levels of HMF were recorded in US for all the biscuits. On the other hand, as expected, BS and US showed higher contents of HMF than the respective HMF content in the whole biscuit. As opposite, MS and LS showed lowest values of HMF as compared with the whole biscuit. Differences of nearly two-fold were found between US and LS fractions. On the other hand, the highest variability in HMF contents was observed in the LS fraction of the biscuits. The results show very huge differences in the baking step whereas heat transfer is not uniformly distributed. This is likely due to the type of oven and the air distribution inside it, as pointed out for colour.

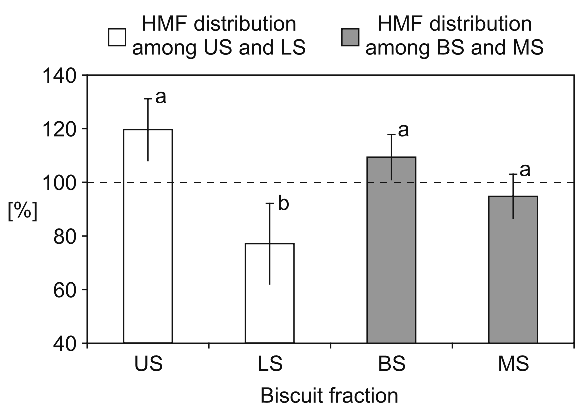

Fig. 2. Mapping of the distribution of HMF in four representative biscuit fractions.

BS – border side, MS – middle side, US – upper side, LS – lower side.

Original mean HMF content in whole biscuits is stated as 100% (dotted line).

Results represent an average of 6 independent samples and error bar represents standard deviation.

Different lower-case letters indicate significant differences (One way Anova and Duncan Test, $P < 0.05$) between US *vs* LS or between BS *vs* MS. No difference between values is designated with the same lower-case letter.

CONCLUSIONS

The determination of HMF in biscuits offers both technological and toxicological information. In the light of our findings, usefulness of HMF to compare different processing conditions is limited since HMF is closely dependent on the dough formulation such as addition of syrup or replacement of reducing saccharides by polyols. However, HMF content is still a useful chemical parameter in the industry for internal quality control for same formulation or processing line. Distribution of HMF inside the biscuits is highly related to the uniformity of the heat load applied during baking. The results of the present study contribute to the challenge of developing a reliable database for HMF levels in foods, as well as the design of in vivo and in vitro studies at realistic concentrations of HMF to evaluate its real impact on human health.

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