Hydroxymethylfurfural contents in foodstuffs determined by HPLC method

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Summary

Hydroxymethylfurfural (HMF; 5-hydroxymethyl-2-furaldehyde) is present in a range of foods containing carbohydrates in an acidic environment. In this work, HMF was determined by the HPLC method in six food commodity groups. The HMF content in tomato purée (n = 11) ranged between 2.8–84.2 mg.kg^{-1}; in ketchups (n = 15) it ranged between 0.8–189.8 mg.kg^{-1}; in syrups (n = 13) and juices (n = 12), it ranged between 0.0–27.3 mg.kg^{-1}; in jams (n = 12) between 4.5–159.6 mg.kg^{-1}, and in fruit baby foods (n = 13), the content of HMF amounted to 2.1– 9.8 mg.kg^{-1}. The total daily exposure to HMF from six investigated food commodity groups for an average person in the Czech Republic may thus amount to 1.11 mg/person/day, of which the exposure to ketchups is the highest (up to 0.54 mg/person/day).

Keywords

HMF; tomato purée; ketchup; fruit juice; jam; fruit baby food; exposure

The technological processes used in food production fundamentally impact on the nutritional and biological value of food and, in most cases, also on its sensory quality. Hydroxymethylfurfural (HMF, 5-hydroxymethyl-2-furaldehyde) is a recognized indicator of reduced quality in numerous foods that contain carbohydrate [1]. This cyclic aldehyde is formed through the dehydration of hexoses and hexuloses in an acidic environment, or as the result of Maillard reactions and caramelisation. In acidic environments, HMF partly decomposes to levulinic acid (4-oxopentanoic acid) and formic acid [2, 3]. However, HMF formation is not limited to foodstuffs with a high content of reducing carbohydrates or proteins. FALLICO et al. [4] have shown a correlation between HMF formation and the content of lipids and their oxidative products in roasted peanuts. ROCHA et al. [5] have even demonstrated HMF formation in the cork stoppers of food glass containers owing to the degradation of their cellulose content due to the technological process.

The presence of HMF in foods is the result of excessive temperatures during heat treatment and inappropriate and long-term storage. HMF reduces the nutritional value of foods [1, 6-13].

Opinions on the cytotoxic, genotoxic and carcinogenic effects of HMF vary [8, 14]. The U. S. Public Health Service, National Toxicology Program has HMF on the list of toxicological studies to be conducted – SCHMIDT and RODRICK [15].

The presence of HMF in honey has been known and monitored for a long time. The Czech legislation (Decree No. 76/2003 Coll. [16], as amended by Decree No. 43/2005 Coll. [17]) sets a maximum to the permitted concentration of this crucial parameter (40 mg.kg^{-1}; this does not apply to honeys from tropical regions and mixtures of such honeys). The same limit value has also been defined in the international legislation for honey, namely by the European Honey Directive [18] and Codex Alimentarius [19].

However, HMF can also be present in other foods containing carbohydrates in an acidic environment, and its concentration can reach considerable values.

The objective of our study was to determine the HMF content in selected vegetable and fruit

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products, available and consumed in the Czech Republic, and to also estimate the average Czech citizen's exposure to HMF from foods in the commodity groups examined. This was calculated on the basis of the HMF content established, and on information about the consumption of the particular foods.

MATERIALS AND METHODS

The samples were bought in the Czech retail network so as to provide a representative spectrum of producers and products present on the Czech market. HMF was determined in a total of 76 samples, including: tomato purées (n = 11), ketchups (n = 15), juices (n = 12), syrups (n = 13), jams (n = 12), and fruit baby foods (n = 13). The juices consisted exclusively of 100 % orange juices. The syrups tested were supplemented with raspberry, orange, strawberry, lemon, blackcurrants, peach, apple, mandarin, forest fruits and garden fruit flavourings. The jams were based on redcurrant, apricot, strawberry, sour cherry, raspberry and peaches. The fruit baby foods (snacks) were based on apples, peaches, raspberries and apples with fruit flakes. All samples were analysed within their minimum shelf-life period.

The samples were prepared using the procedure as defined by RADA-MENDOZA et al. [9]. Two parallel analyses were carried out on each sample. Prior to analysis the samples were homogenised by stirring or mixing, and the pH value was measured using the ROSS combination pH electrode or the ROSS combination spear-tip pH electrode (Orion Research, Beverly, USA) depending on the sample consistency, using Orion pH meter model 250 A (Orion Research). Approximately 1 g of the homogenised sample, in the form of a water suspension or solution, was transferred into a volumetric flask of 25 ml. After thorough mixing, 2 ml of Carrez I and 2 ml of Carrez II solutions were added to the flask and the volume was topped up with ultra-clean water to 25 ml. After 30 minutes, the supernatant was filtered through a 0.45 μm membrane filter (Millipore, Billerica, USA) and analysed by means of HPLC.

HPLC determination

The chromatographic determination was carried out on an Alliance apparatus manufactured by Waters Company, with a 2996 diode array detector. Nova Pack C 18, 150 x 3.9 mm, 4 μm column was used (Waters, Milford, USA).

The isocratic HPLC system was used for the analysis, water + methanol (90 : 10) were used as the mobile phase, all HPLC grade. The mobile phase flow rate was 1 ml.min^{-1}, with the sample injection volume of 20 μl and the column temperature of 25 °C. HMF was detected in the UV region at 285 nm.

Validation of parameter determination

The external standard method was used for the determination of the HMF content using the Empower software (Waters). The linearity of the HPLC method used was tested in the concentration range of 0.01–200 mg.l^{-1} by means of an HMF standard (Sigma, Milwaukee, USA). Reproducibility and recovery were tested by the standard addition method on two types of matrices, namely fruit baby food and tomato purée. The detection limit of the method used was determined as 0.03 mg.kg^{-1}. The reproducibility of the method, expressed as relative standard deviation, ranged from 0.9 to 2.6 % for the individual matrices. The recovery of the method was determined by adding a known amount of the standard at concentration levels of 10; 60 and 100 mg.kg^{-1}. This amounted to 85 % for the ketchup and baby food matrices, 105 % for juices, 102 % for tomato purées and 93 % for jams.

Statistical evaluation of the results

The HMF content data were statistically evaluated using basic statistical variables and Microsoft Office Excel 2003 (Microsoft, Redmond, USA). The possible dependence of the HMF content on pH values and on carbohydrates content was tested (for two commodities) by determining the respective correlation coefficients using STAT Plus software [20].

RESULTS AND DISCUSSION

In the analysed samples of vegetable and fruit products, the HMF content found ranged from effectively zero values (i. e. below the detection limit of the method used) up to 189.9 mg.kg^{-1}.

As expected, the lowest HMF values were measured for fruit juices; in four samples, the concentrations were below the detection limit. The highest value measured only amounted to 2.8 mg.kg^{-1}.

A very low HMF concentration was also established in syrups (8.7 ± 7.63 mg.kg^{-1}). In fruit baby foods, the HMF cocentration was very low as well, only in the order of milligrams (7.2 ± 2.47 mg.kg^{-1}). However, the average values

Tab. 1. HMF concentrations in the analysed samples.

Commodity	n	HMF [mg.kg-1]				
		x_{min}	x_{max}	median	x	s_x
ketchup	15	0.8	189.9	11.1	26.5	45.73
tomato purée	12	2.8	84.2	46.8	45.7	25.81
fruit baby food	13	2.1	9.8	8.0	7.2	2.47
jam	12	4.5	159.6	20.0	37.2	41.87
fruit juice 100%	12	0.0*	2.8	0.1	0.4	0.75
syrup	13	1.3	27.3	6.6	8.7	7.63

n - number of samples, x_{min} - minimum value, x_{max} - maximum value, x - average, s_x - standard deviation, * - below the detection limit of the method used.

obtained were higher than those reported by Rada-Mendoza et al. [9], who obtained the average value of 2.9 mg.kg^{-1} for this commodity.

The situation was different with ketchups and jams. Although the average value for ketchups amounted to 26.5 ± 45.73 mg.kg^{-1}, one sample contained as much as 189.9 mg.kg^{-1} HMF. This commodity group showed the largest spread of values (0.8–189.9 mg.kg^{-1}). The situation was similar regarding jams: the average values of HMF were 37.2 ± 41.87 mg.kg^{-1}, i. e. more than that measured for ketchups. The HMF values measured by us were higher than those reported by Rada-Mendoza et al. [9], who measured 71.7 mg.kg^{-1} as the highest value, with the average being 13.5 mg.kg^{-1}. Taking 50 mg.kg^{-1} HMF as the upper limit for the assessment of the adequate technological conditions of the production of jams, as proposed by Steber and Klostermeyer [21], this limit was exceeded by 3 samples.

The highest average value of all the commodity groups examined was measured for tomato purées: 45.7 ± 25.81 mg.kg^{-1}. Table 1 presents an overview of the HMF data along with the basic statistical parameters for the individual groups of foods.

It is obvious that, owing to heat processing which is necessary for the requisite minimum shelf life, HMF builds up more in ketchups, jams and tomato purées. The higher temperatures result in Maillard reactions and caramelisation, particularly in the conditions of high carbohydrate content and low pH values [9]; in jams, these reactions are also enhanced by the low water activity value.

In order to establish the dependence of the HMF concentration on carbohydrate content and pH value, the pH values were measured, and the carbohydrate content was recorded for two commodity groups (baby foods and jams) on the basis of the manufacturer's data shown on the product (Tabs. 2 and 3). A correlation analysis of HMF content vs. pH values, and/or of HMF content vs. carbohydrate content in fruit baby foods gave correlation coefficients of $r = -0.48$ (HMF content vs. pH) and $r = 0.55$ (HMF content vs. carbohydrate content). For jams, the values of $r = 0.21$ (HMF vs. pH) and $r = 0.12$ (HMF vs. carbohydrate content)

Tab. 2. HMF contents and other parameters of jam samples.

Jam	HMF [mg.kg-1]	Carbohydrates [%]	pH
strawberries	22.8 ± 0.2	60	3.06
mixture	45.3 ± 0.9	60	2.82
strawberries	159.6 ± 4.4	60	3.24
apricots	61.8 ± 2.1	60	2.57
sour cherries	8.7 ± 0.2	43	3.03
apricots	4.5 ± 0.1	60	2.76
strawberries	22.4 ± 0.7	65	3.06
peaches	5.6 ± 0.1	60	3.04
mixture	15.4 ± 0.2	60	2.84
raspberries	17.5 ± 0.0	53	3.23
currants	65.6 ± 6.1	60	2.74
strawberries	17.5 ± 0.3	60	3.05

Tab. 3. HMF contents and other parameters of baby food samples.

Samples	HMF [mg.kg-1]	Carbohydrates [%]	pH
1	6.25 ± 0.11	19.9	3.38
2	4.97 ± 0.03	14.2	3.39
3	9.57 ± 0.03	19.9	3.38
4	2.06 ± 0.07	11.2	3.40
5	9.38 ± 0.12	19.9	3.40
6	6.01 ± 0.02	15.4	3.40
7	9.08 ± 0.05	19.9	3.38
8	9.39 ± 0.12	19.9	3.39
9	7.97 ± 0.06	19.9	3.40
10	7.47 ± 0.08	19.9	3.40
11	9.76 ± 0.08	19.9	3.38
12	8.15 ± 0.23	9.5	3.39
13	2.88 ± 0.04	17.2	3.40

were obtained. The correlation coefficients were statistically insignificant. It may thus be concluded that above all non-observance of the heat treatment standards in the production rather than carbohydrate content or pH value of the food are the limiting factors for HMF formation.

The average exposure of an average Czech citizen from the foods from the commodity groups examined can be estimated from the established values of the HMF content in the different foods and from the consumption of these foods (reported by Ruprich et al. [22]). Table 4 shows the consumption of the tested foods in grams per person per day, and the calculated average and maximum exposure to HMF at the given consumption rates. The total daily exposure of an average person can thus amount up to 1.11 mg/person/day, of which the exposure from ketchups is the highest, being up to 0.54 mg/person/day (Tab. 4).

Honey is the only food for which a legal limit on HMF concentration has been set. In our former study, honeys obtained directly from beekeepers after extraction were shown to contain very low HMF concentrations (10.0 ± 9.0 mg.kg-1). However, at the end of their shelf-life, 55.6 % of samples exceeded the limit of 40 mg.kg-1 permitted by law [11].

Assuming an HMF intake from honey of 1.385 g/person/day in the Czech Republic [22], with the maximum permitted content being 40 mg.kg-1, then the maximum exposure is 0.0554 mg/person/day.

The results of our study suggest that, apart from honey, also a number of other food commodities, including those based on fruit and vegetables, available in the retail network and consumed in the Czech Republic, contain HMF.

The normal levels of HMF in foodstuffs are not associated with any risk to the general population. However, attention should be paid to particular groups of sensitive people, especially children, for whom increased HMF intake could present certain risks. Children consume large amounts of many of the foods which have ideal conditions for the formation of HMF (in addition to baby foods, this also includes marmalades, fruit juices, syrups, milk,

Tab. 4. Estimated exposure to HMF from foods in the commodity groups analysed.

Commodity	Consumption [g/person/day]	Average exposure [mg/person/day]	Maximum established exposure [mg/person/day]
ketchup	2.83	0.075	0.537
tomato purée	0.40	0.018	0.034
fruit baby food	0.40	0.003	0.004
jam	0.80	0.030	0.128
fruit juice 100%	33.73	0.014	0.094
syrup	11.27	0.087	0.308
Total	50.82	0.226	1.105

honey and ketchups). We may thus recommend that HMF content in foods be monitored, and setting of certain limits in respect of the sensitive groups should be considered and the public should be kept informed (e.g. on product packaging).

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