ABSTRACT
5-Hydroxymethyl-2-furfural (5-HMF) is a thermal decomposition product of saccharides. There are two main ways for the formation of 5-HMF. First, 5-HMF is forming during Maillard reaction and second, during thermic dehydration of saccharides under acid conditions. Significant parameters of 5-HMF formation are temperature, time, pH, water activity, type of saccharide and amino acids. It is suspected that 5-HMF has genotoxic, mutagenic and carcinogenic potential. This chemical can be found in many food sources, e.g. honey, dried fruits, fruit juice and concentrates, alcoholic beverages, bakery products, roasted nuts and seeds, brown sugar, and milk.

The present study aimed to determine the amount of 5-HMF in children’s biscuits. The examined samples were divided into three groups. The first group of biscuits claimed availability for children older than six months, the second for children older than one year. The third group did not give a determined age range.

For the assessment of 5-HMF, a HPLC method with UV/VIS detection was used. In the first group of samples, the amount of 5-HMF ranged from 0.34±0.04 to 1.73±0.03 mg/kg, in the second group from 0.57±0.09 to 1.78±0.07 mg/kg, and in the third group the amounts of 5-HMF were from 1.80±0.05 to 34.99±0.22 mg/kg.

In conclusion, the results showed that the content of 5-HMF in biscuits without age group determination was significantly higher than in biscuits with declared availability for children older than six months or one year. Since the acceptable daily intake is 2 mg/kg bw, the established amount of 5-HMF in all samples cannot be regarded as dangerous in a normal dose of biscuits.

KEY WORDS: 5-hydroxymethylfurfuraldehyde; biscuits; HPLC; children

Introduction
Thermal treatment is commonly used as a way of preparation of food products. Cooking, baking, roasting, extrusion cooking, pasteurization or sterilization, are the most frequently used processes. With thermal processing, there are deeply wedded reactions, which are needed to form aroma, taste and color. For some food products like milk or fruit juices, it is necessary to eliminate the impact of thermal treatment as much as possible to keep them natural, with fresh appearance and optimal nutrition quality. On the other hand, thermal treatment is a compulsory measure to achieve required properties of some food products (Kowalski et al., 2013). These products include for example: bakery products (Petisca et al., 2014), cereals (Delgado-Andrade et al., 2008), cocoa (Sacchetti et al., 2016), coffee (Arribas-Lorenzo & Morales, 2010), fruit juices (Lee et al., 2014), jams and fruit-based infant food (Rada-Mendoza et al., 2002), as well as brown sugar (Polovková & Šimko, 2017). During thermal treatment several reactions are in progress, as e.g. Maillard reaction, caramelization, lipid oxidation. Due to these reactions, from heating processes arise not only substances with positive effects on our senses and health, but also contaminants are produced. One of these contaminants is furanic compound 5-hydroxymethyl-2-furfural (5-HMF) (Capuano & Fogliano, 2010).

There are two main ways for the formation of 5-HMF. First it is caramelization of sugars under thermal treatment and acid catalysis. In this way, sucrose can be decomposed to free fructose and fructofuranosyl cation. When this reaction is in progress at a temperature above 250 °C and under dry pyrolitic conditions, fructofuranosyl cation can be converted to 5-HMF (Nguyen et al., 2016). The second way of formation of 5-HMF is Maillard reaction (MR). The free amino group of lysine and/or other amino acids and the carbonyl groups of reducing saccharides, such as glucose or maltose, enter into the MR as precursors (Delgado-Andrade et al., 2009). The type and concentration of sugars, amino acids, temperature, time, pH, water activity, leaving agents, antioxidants, content
of NaHSO₃ and vitamin E are all parameters which influence to formation of 5-HMF (Nguyen et al., 2016).

High concentrations of 5-HMF have been shown to cause cytotoxicity, irritability of eyes, upper respiratory tract, skin and mucous membranes (Capuano & Fogliano, 2010). Studies on rats and mice indicated that 5-HMF can be potentially carcinogenic (Kowalski et al., 2013). Moreover, during the metabolic process 5-HMF is converted to 5-sulfoofymethyl-2-furfuraldehyde (SMF) (Van Der Fels-Klerx et al., 2014). Sulphate located in SMF is a good leaving group that can produce a highly reactive intermediate and can react with DNA (Capuano & Fogliano, 2010).

It is yet not clear if 5-HMF represents a potential health risk for humans by dietary exposure. Janzovski et al. (2000) assumed that even though the highest concentration of 5-HMF in some food products is near the biologically effective concentrations, the authors claim that average food 5-HMF exposure should not represent a health risk.

The aim of this study was to determine the amount of 5-HMF in biscuits marketed in Slovakia and establish the difference between biscuits which declare availability for children older than six months or one year and biscuits without specified age range.

Materials and methods

Chemical and reagents

Analytical standard of 5-HMF was supplied by Sigma-Aldrich (Darmstadt, Germany), acetonitrile was purchased by Fisher chemicals (Loughborough, UK). Potassium hexacyanoferrate trihydrate was supplied by Merck (Darmstadt, Germany) and zinc sulphate heptahydrate by Honeywell Fulka (Seelze, Germany).

Samples

Thirteen samples of biscuits were obtained from Tesco Stores SR. Four samples were biscuits with declared availability for children older than 6 months, four samples with availability for children older than 1 year and five samples without determined age range.

Extraction of 5-HMF from biscuits

Samples of biscuits were powdered in a grinder and homogenized. Three grams of the sample were put into a 100 ml volumetric flask and consequently 50 ml of deionized water was added. The flask with the sample and water was put into ultrasonic bath for 15 minutes. Then 1 ml of Carrez I (15 % solution of K₄[Fe(CN)₆]) and 1 ml of Carrez II (20 % solution of ZnSO₄) were added and the flask was filled with deionized water to 100 ml.

Analysis of 5-HMF

5-HMF was analyzed by using Agilent 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA) with UV/VIS detector set at 285 nm. Aliquots of samples were injected manually in the volume of 25 μl. Agilent Zorbax Reliance Cartridge Guard-column and Zorbax Eclipse Plus C18 4.6×100 mm column was used. The mobile phase consisted of a mixture of water/acetonitrile in the ratio 90:10 for preservation isocratic conditions. The time of analysis was set at 10 minutes and 5-HMF eluted in 2.7 minute.

Results and discussion

Sample description

The main ingredients in biscuits are cereal flour, saccharides and fats. A dough is conventionally baked at high temperature (up to 200 °C) and in a short time (<20 min). Finally, the effect of baking is low content of water and brown surface of biscuits (Ait-Amoure et al., 2006). For our analysis, semi-sweet biscuits without fillings were chosen. Table 1 summarizes the main information about the macronutrients and energy. All samples had similar levels of major constituents. The greatest difference was only in the content of fiber and proteins.

Sample treatment and analysis

5-HMF has good solubility in water and extraction from samples is relatively good. Ultrasonic bath was used to accelerate and maximize extraction and Carrez solutions were used for clarification of sample solutions. Mobile phase for HPLC analysis was prepared according to Ramírez-Jiménez et al. (2000) and adapted to our lab conditions. Every sample was analyzed three times and accuracy of peak was verified by adding standard solution of 5-HMF into the sample.

Content of 5-HMF in biscuits

The content of 5-HMF in samples of biscuits suited for children older than six months ranged from 0.34±0.04 to 1.73±0.03 mg/kg, for older than one year from 0.57±0.09 mg/kg.

Table 1. Summary of the energy, protein, carbohydrate, fat, fiber and salt in 100 g of samples declared by the manufactures.

| Group/Nr. of sample | Energy [kJ] | Protein [%] | Carbohydrate [%] | Fat [%] | Fiber [%] | Salt [%] |
|---------------------|------------|-------------|------------------|--------|-----------|---------|
| BS2/1               | 1770       | 9.9         | 65.6             | 13.4   | 0         | 0.57    |
| BS1/1               | 1795       | 11.2        | 68.9             | 10.9   | 3.8       | 0.88    |
| BS2/2               | 1790       | 10.7        | 69.1             | 10.9   | 3.8       | 0.875   |
| BS2/3               | 1770       | 9.9         | 65.6             | 13.4   | 0         | 0.57    |
| BS1/3               | 1869       | 7           | 75               | 13     | 1.9       | 0.48    |
| BS3/1               | 1779       | 10.4        | 72.1             | 9.8    | 1.7       | 0.63    |
| BS3/2               | 1898       | 5.8         | 75               | 14     | 0         | 0.5     |
| BS3/3               | 1791       | 6           | 75.8             | 10.4   | 2         | 0.41    |
| BS3/4               | 1896.2     | 6.23        | 72.66            | 15.52  | 2.13      | 0.05    |
| BS3/5               | 1736.44    | 12.66       | 71.45            | 8.76   | 7.8       | 0.07    |
| BS3/6               | 1871.06    | 7.46        | 71.51            | 14.76  | 1.95      | 0.02    |
| BS3/7               | 1894.23    | 8.83        | 70.38            | 15.31  | 2.09      | 0.02    |
| BS3/8               | 1893       | 9           | 73               | 13     | 2.3       | 0.38    |

Group BS1 represents biscuits with availability for children older than six months; group BS2 biscuits for children older than one year; BS3 are biscuits without age specifications.
to 1.78±0.07 mg/kg and in samples without age specifications it ranged from 1.80±0.05 to 34.99±0.22 mg/kg. Results of the analysis are depicted in Figure 2 and given from lowest to highest amount of 5-HMF. The biscuits intended for children older than six months and one year have a lower concentration of 5-HMF as compared to samples without age specifications.

The highest amount of 5-HMF was found in the sample BS3/5. This marked difference could be caused by a sweetener used in the biscuit recipe. This sample consisted of invert sugar syrup as a sweetener compared to sucrose used in other biscuits. Invert sugar syrup is a mixture of glucose and fructose. Nguyen et al. (2016) proved that 5-HMF rise more rapidly from glucose and fructose than from sucrose, possibly explaining this difference.

The determined content of 5-HMF in our samples was in concordance with the results of Ait-Ameur et al. (2006) who measured the content of 5-HMF in biscuits marketed in France (ranging from 0.5 mg/kg to 78.6 mg/kg) and Petisca et al. (2014) who measured the amount of 5-HMF in the range from 1.65 mg/kg to 82.78 mg/kg. Delgado-Andrade et al. (2009) reported a 5-HMF content from 3.1 mg/kg to 182.5 mg/kg. The much higher maximal values in the above studies compared to our samples were probably caused by selection of biscuits for children in our case.

For producers it is important to find the optimal ratio of all factors which can influence the health quality of biscuits, their properties like structure, size, taste, aroma, color and also cost. Finding this ratio is not simple, but even though many factors influence the amount of 5-HMF in biscuits, there are manufacturers who are able to produce biscuits with minimal amount of 5-HMF.

Our results indicate a higher quality of biscuits with availability for children older than six months or one year. It is necessary to emphasize, that 5-HMF can originate from several sources (bakery products, chocolate, milk,
fruit processed products, etc.) and these might contribute to the overall exposure to 5-HMF. Although we found a high concentration range of 5-HMF in biscuits for children, we do not see a health risk since the amount needed to achieve acceptable the daily intake concentration for a 20kg child is about 0.5 kg of biscuits. However, attention is demanded for the daily diet since quite a large number of food products contain 5-HMF.

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REFERENCES

Ait-Ameur L, Trystram G, Birlouez-Aragon I. (2006). Accumulation of 5-hydroxymethyl-2-furfural in cookies during the backing process: Validation of an extraction method. Food Chemistry 98(4): 790–796.

Arribas-Lorenzo G, Morales FJ. (2010). Estimation of dietary intake of 5-hydroxymethylfurfural and related substances from coffee to Spanish population. Food Chem Toxicol 48(2): 644–649.

Capuano E, Fogliano V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT - Food Sci Tech 44(4): 795–810.

Delgado-Andrade C, Seiquer I, Navarro MP, Morales FJ. (2008). Estimation of hydroxymethylfurfural availability in breakfast cereals. Studies in Caco-2 cells. Food Chem Toxicol 46(9): 1600–1607.

Delgado-Andrade C, Rufián-Henares JA, Morales FJ. (2009). Hydroxymethylfurfural in commercial biscuits marketed in Spain. J Food Nutrition Res 48(3): 14–19.

Gökmen V, Acar J. (1999). Simultaneous determination of 5-hydroxymethylfurfural and patulin in apple juice by reversed-phase liquid chromatography. J Chromatogr A 847(1–2): 69–74.

Janzowski C, Glaab V, Samimi J, Schlatter J, Eisenbrand G. (2000). 5-hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. Food Chem Toxicol 38: 801–809.

Kowalski S, Lukasiewicz M, Duda-Chodak A, Zięc G. (2013). 5-Hydroxymethyl-2-furfural (HMF)-heat-induced formation, occurrence in food and biotransformation – a Review. Polish Journal of Food and Nutrition Science 63(4): 207–225.

Lee TP, Sakai R, Manaf NA, Rodhi AM, Saad B. (2014). High performance liquid chromatography method for the determination of patulin and 5-hydroxymethylfurfural in fruit juices marketed in Malaysia. Food Control 38: 142–149.

Nguyen HT, Van der Fels-Klerx HJ (Ine), Peters RJB, Van Boekel MAJS. (2016). Acrylamide and 5-hydroxymethylfurfural formation during baking of biscuits: Part I: Effects of sugar type. Food Chemistry 192: 575–585.

Petisca C, Henriques AR, Pérez-Palacios T, Pinho O, Ferreira IMPVLO. (2014). Assessment of hydroxymethylfurfural and furfural in commercial bakery products. J Food Compos Anal 33(1): 20–25.

Polovková M, Simko P. (2017) Determination and occurrence of 5-hydroxymethylfurfural in commercial jams and in fruit-based infant foods. Food Chemistry 79(4): 513–516.

Rada-Mendoza M, Olano A, Villamil M. (2002). Determination of hydroxymethylfurfural in commercial jams and in fruit-based infant foods. Food Chemistry 79(4): 513–516.

Ramírez-Jiménez A, García-Villanova B, Guerra-Hernández E. (2000). Hydroxymethylfurfural and methylfurfural content of selected bakery products. Food Res Int 33(10): 833–838.

Sacchetti G, Iannone F, De Gregorio M, Di Mattia C, Serafini M, Mastrocola D. (2016). Non enzymatic browning during cocoa roasting as affected by processing time and temperature. J Food Eng 169: 44–52.

Van der Fels-Klerx HJ, Capuano E, Nguyen HT, Aţaş Mogol B, Kocadağlı T, Gönçüoğlu Taş N, Hamzálioğlu A., Van Boekel M.A.J.S., Gökmen V. (2014). Acrylamide and 5-hydroxymethylfurfural formation during baking of biscuits: NaCl and temperature-time profile effects and kinetics. Food Res Int 57: 210–217.