

**Original Article****Identification and Quantification of 5-Hydroxymethylfurfural in Food Products**Maryam Jalili^{*1,2}, Farzaneh Ansari¹

1-Dept. of Food Industries and Agricultural Research, Standard Research Institute (SRI), Karaj, Iran

2- Faculty of Health and Nutrition, Lorestan university of Medical Science, Lorestan, Iran

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Background and Objectives: 5-Hydroxymethylfurfural (5-HMF) is known as an indicator of quality deterioration in a wide range of foods. The current study covered 70 samples taken from domestically produced foods and drinks available on the Iranian market (including honey, jam, fruit cakes tomato paste, ketchup, syrup, fruit juice, canned fruit, ultra-high-temperature (UHT) milk, instant coffee, and jelly powder).

Materials and Methods: HMF levels were determined using high performance liquid chromatography (HPLC) and ultraviolet (UV) detector.

Results: The mean recovery values ranged from 84.4 to 105.8%. Varying amounts (11.42-929 mg kg⁻¹) of HMF were found in 48 out of the 70 (87%) samples analyzed. High levels of HMF were mainly seen in commercial honey (20.55–928.96 mg kg⁻¹), jams (51.10 to 245.97 mg kg⁻¹), fruit cakes (nd-171.50 mg kg⁻¹), and ketchup (32.70-72.19 mg kg⁻¹). No HMF content was detected in UHT milk, instant coffee, and jelly powder.

Conclusions: The results of our study suggest that, apart from honey, a number of other food commodities, including those containing fruit and/or sugar, hold considerable amounts of HMF. Therefore monitoring of HMF levels in foods seems to be necessary.

Keywords: Hydroxymethylfurfural (HMF), Honey, High performance liquid chromatography

Introduction

Food products are subjected to thermal treatments to obtain desirable sensory properties or texture features, assure microbiological safety, and eliminate enzymatic activities (1). 5-Hydroxymethylfurfural (HMF) is one of most known Amadori compounds formed during the thermal treatments of carbohydrate-containing foods as a result of Maillard reaction (the non-enzymatic browning reaction) and caramelization. Therefore, HMF generally is known as an indicator of quality deterioration as a result of excessive heating or storage in a wide range of foods (2). The following factors influence the formation of HMF in foods: (a) carbohydrate content, (b) physicochemical properties (pH, total acidity), (c) thermal treatment, (d) water activity, (e) long-term storage, and (f) use of metallic containers (1). In general, monosaccharides (*i.e.* fructose or glucose) are the basic substrates for HMF production. Moreover, disaccharides and most polysaccharides

are hydrolyzed into simple sugars, which, subsequently, act as starting material for HMF formation. The process also may be strongly enhanced under acidic conditions in the absence of amino groups (3). In acidic medium, HMF is formed by decomposition of hexoses during the heating after a slow enolization and a fast β -elimination of three water molecules (4). Therefore, foods containing simple sugars and acids, namely honey, jam, cereal products, and fruit and vegetable products provide more suitable media for the formation of HMF. Although HMF is a by-product of thermal processing, its impact on human health is still a contentious topic. There is much debate over its toxicity, genotoxicity, mutagenicity, and carcinogenicity (1). Some authors believe that HMF is a natural component of traditional foods, posing no risk to human health (5), while others believe that HMF can act as a neurotoxin and consequent to accumulation in the body and in

*Address for correspondence: Maryam Jalili, Department of Food Industries and Agricultural Research, Standard Research Institute (SRI) Karaj, Iran, and Faculty of Health and Nutrition, Lorestan university of Medical Science, Lorestan, Iran. Tel: (+98 26) 32861128; E-mail address: Jalili@standard.ac.ir

combination with proteins eventually lead to lesions in muscles and the viscera (6). Durling et al. reported significant HMF-induced DNA damage after 3 hours of exposure to 100 mM HMF. They reported that HMF is a DNA damaging agent; however, damage was only observed at rather high concentrations (7). Moreover, HMF can be metabolized to 5-sulfoxy methylfurfural (SMF), a reactive intermediate that can bind to DNA and cause mutagenic effects. Both HMF and SMF are weak intestinal carcinogens in mice (8).

From a safety perspective, HMF is produced in large quantities, and the levels can exceed 1g/kg in several food items; however, honey is the only food for which a legal limit on HMF concentrations has been set (9). The Codex Alimentarius (10) has established that after processing and/or blending, the HMF content of honey must not exceed 80 mg kg⁻¹. The European Union (EU) recommends a lower limit of 40 mg kg⁻¹ with the following exceptions: 80 mg kg⁻¹ is allowed for honey that originates from countries or regions with tropical temperatures (11). The legal limit of 40 mg kg⁻¹ had already been issued for honey by the Institute of Standards and Industrial Research of Iran (ISIRI) (12).

Given the fact that HMF is usually a recognized parameter indicating the freshness and quality of foods, some researchers have determined the amount of HMF in some kinds of food including bread (13), coffee (14), honey (15, 16, 17, 18), fruit juice (15, 18), raisins (19), milk (20), instant coffee (21), biscuits, jam, and breakfast cereal (15).

The objective of the current study is to determine the HMF content in different kinds of domestically produced foods and drinks available on the Iranian market. In case of honey and jam, the health risks are also assessed.

Materials and Methods

Samples: A total of 70 samples of local foods and drinks were collected randomly from different supermarkets, located in the cities of Tehran and Karaj (Iran) during April to September 2013. The samples were purchased in commercially available size to obtain about 1.0 kg of each sample. The collected samples included honey (n=16), jam (n=10), fruit cake (n=10), tomatoes paste (n=4), ketchup (n=5), syrup (n=4), fruit juice (n=8), canned fruit (n=8), sterilized milk (n=2), instant coffee (n=2) and

jelly powder (n=1). The samples were analyzed immediately after being purchased; otherwise, they were stored at -20°C before being analyzed.

Chemicals and reagents: HPLC-grade water was obtained from a water purifier (Elga, Marlow, Buckinghamshire, UK). HMF, methanol, potassium hexacyanoferrate (II) (K₄Fe(CN)₆·3H₂O), zinc acetate (ZnCH₃COO)₂·2H₂O and disodium hydrogen phosphate (Na₂HPO₄) were supplied by Merck (Darmstadt, Germany). Filter paper was purchased from Schleicher & Schuell Microscience GmbH (Dassel, Germany). Stock solution of 2 mg mL⁻¹ HMF was prepared by dissolving 10 mg in 5 mL de-ionized water in a plastic volumetric flask. Working standards at lower HMF concentrations were prepared by diluting appropriate volumes of the stock standard solution with de-ionized water. The standard solutions were kept in plastic containers at 4 °C when not in use.

Method validation: In the assessment of linearity, a seven point calibration of HMF curve was constructed. Proper amounts of the working standard solution of HMF were diluted to obtain the final concentrations of 2, 5, 10, 20, 40, 70 and 100 ng mL⁻¹. Calibration curve was obtained using the linear least squares' regression procedure of the peak area versus the concentration. With regard to the accuracy of the method applied and since there was no certified reference material (CRM) available, HMF-free samples, spiked with respective HMF levels of 5.0, 50.0 and 100.0 mg kg⁻¹ (n=5). Recovery experiments were performed on honey, jam, fruit cake, ketchup, syrup, fruit juice, canned fruit and ultra-high-temperature (UHT) milk. Repeatability for HMF was expressed as relative standard deviation (RSD). The limit of detection (LOD) and limit of quantitation (LOQ) were determined by using the signal-to-noise approach, defined as the concentration that results in a signal-to noise ratio of approximately 3:1 and 10:1 for LOD and LOQ, respectively.

Determination of HMF content: The HPLC method used was based on the method published by the German Institute for Standardization (22). In brief, at first, the samples were mixed thoroughly; 10 g of each sample was placed in a 100 ml conical flask diluted to 50 ml with distilled water. Then, 1 ml of a 15% (w/v) Carrez I (potassium hexacyanoferrate) solution and 1 ml of a 30% (w/v) and 1 ml of Carrez

II (zinc acetate dehydrate) solution were added to clarify the samples, and made up to 100 ml with distilled water. The solution was filtered through Whatman filter paper. After discarding the first 5 ml, the filtrate was passed through a 0.45 μm nylon membrane filter. A total of 50 μl of the filtered sample elute was injected to the HPLC for HMF analysis. The HPLC system (Varian 9010, Creek, California, USA) was equipped with a Knauer degasser (Berlin, Germany), and a variable wavelength ultraviolet (UV) Detector (Varian 9050, Creek, California, USA). The HPLC column was an Agilent Bondesil, RP-C18, (4.6 mm, 5 μm , 25cm). The mobile phase used during the HPLC analysis consisted of 95% water (0.01 Mol/L) and 5% methanol with a flow rate of 1.0 ml/min. The detection wavelength was 284 nm. The HMF content of the sample was calculated by comparing the corresponding peak areas of the sample and those of the standard solutions of HMF (Sigma–Aldrich, USA) after correcting for dilution.

Statistical analysis: The HMF content data were statistically evaluated using basic statistical variables. To present the basic features of the data in the current study, values of all samples are presented as mean \pm SD, minimum and maximum by applying Minitab (Version 13.2, State College, PA., USA). According to the legal limit of HMF issued for honey by ISIRI (12), the data were presented in two different ranges, less and more than 40 mg kg^{-1} .

Results

Method validation: The calibration curve obtained by least-squares' linear regression was linear with the correlation coefficient of 0.998. The mean recovery values ranged from 84.4 to 105.8 for spiking of canned fruit cake and honey at 5.0 and 100 mg kg^{-1} , respectively, as summarized in Table 1. The RSD was found to be between 3.3% to 7.5% for spiking of milk and canned fruit at 100 and 50 ng g^{-1} , respectively (Table1). The LOD and LOQ were 0.02 and 0.06 mg kg^{-1} , respectively.

Table 1: Recovery and RSD obtained for HMF determination in blank matrix of honey, jam, fruit cake, ketchup, syrup, fruit juice, canned fruit and UHT milk at three different spiking levels (n = 5)

Sample	Spiking level (mg L^{-1})	Mean recovery \pm SD	RSD %
Honey	5	96.1 \pm 5.8	6.1
	50	104.8 \pm 4.7	4.4
	100	105.8 \pm 7.8	7.4
Jam	5	97.2 \pm 3.6	3.7
	50	93.6 \pm 5.5	5.8
	100	95.6 \pm 6.1	6.3
Fruit cake	5	97.8 \pm 7.1	7.3
	50	94.2 \pm 6.9	7.3
	100	86.0 \pm 5.5	6.4
Ketchup	5	94.4 \pm 4.5	4.8
	50	94.7 \pm 5.6	5.9
	100	97.7 \pm 6.3	6.5
Syrup	5	92.3 \pm 5.5	5.9
	50	93.7 \pm 5.2	5.6
	100	96.9 \pm 4.6	4.8
Fruit juice	5	92.7 \pm 4.6	5.0
	50	94.9 \pm 4.7	4.9
	50	100.4 \pm 7.1	7.1
Canned fruit	5	84.4 \pm 5.3	6.3
	50	86.6 \pm 6.5	7.5
	100	96.6 \pm 7.0	7.3
UHT Milk	5	97.2 \pm 6.7	6.9
	50	101.0 \pm 5.7	5.7
	100	104.4 \pm 3.5	3.3

RSD: Relative standard deviation for repeatability

Incidence: Seventy different samples of foods and drinks were analyzed; some of them contain sugar (honey, jam, fruit cakes, ketchup, syrups, canned fruits, jelly powder, and fruit juice) and some lack sugar (UHT milk, tomato paste and instant coffee). The mean, minimum and maximum HMF concentrations of all samples are listed in Table 2. The permitted limit on HMF concentration levels has been set only for honey (40 mg kg⁻¹); therefore, in the current study, the HMF contamination range of samples is compared with the 40 mg kg⁻¹. Based on the HPLC method, variable amounts of HMF (11.42-929 mg kg⁻¹) were found in 61 out of the 70 (87%) analyzed samples. Sixteen honey samples showed expanded range of HMF concentration from 28.79 to 928.96 mg kg⁻¹. The HMF content of 10 analyzed samples of jam (apple, apricot, carrot, black cherry, pumpkin, and mango), regardless of the pH, sugar,

kind of fruit, dried matter or other specifications, ranged from 51.10 to 245.97 mg kg⁻¹ (Table 2). In the analyzed samples of tomato products, 4 out of 5 ketchup samples (80%) showed HMF levels higher than 40 mg kg⁻¹, while lower HMF levels were found in tomato paste. Lower HMF values (below 40 mg kg⁻¹) were obtained for 8 fruit juice samples (orange, apple, and pineapple juice), ranging from 11.42 to 39.24; and those of 8 canned fruit samples (apple, pineapple and apricot) ranged from 15.74 to 44.32 mg kg⁻¹ (Table 2). A relatively higher HMF concentration level was also established in 4 syrup samples (47.73 to 63.20 mg kg⁻¹). The results showed that 50% of the samples contained HMF levels higher than 40 mg kg⁻¹. The highest value measured amounted to 71.50 mg kg⁻¹. No HMF content could be detected in UHT milk, instant coffee and jelly powder.

Table 2: Occurrence of HMF in 70 food and drink samples determined using HPLC-UV

Sample	No. of samples	Positive (%)	Mean±SD	Minimum	Maximum	n.d-40 mg kg ⁻¹	> 40 mg kg ⁻¹
Honey	16	16 (100%)	161.00±236.88	20.60	929.00	6	10
Jam	10	10 (100%)	97.80±59.99	48.90	246.00	-	10
Fruit cake	10	7 (70%)	47.87±56.27	n.d	171.50	5	5
Tomato paste	4	3 (75%)	12.59±8.64	n.d	19.54	4	-
Ketchup	5	5 (100%)	57.31±18.64	32.70	79.15	1	4
Syrup	4	4 (100%)	54.89±6.53	47.73	63.20	-	4
Fruit juice	8	8 (100%)	23.21±14.08	11.42	39.24	8	-
Canned fruit	8	8 (100%)	25.14±9.85	15.74	44.32	7	1
UHT Milk	2	n.d					
Instant coffee	2	n.d					
Jelly powder	1	n.d					

Discussion

Method validation: The linearity in the working standard solutions of six concentration levels was reliable. The values obtained for recovery, LOD and LOQ were comparable with the previous results. Vorlova and co-workers reported that the detection limit of the method used for determination of HMF was 0.03 mg kg⁻¹, and the recovery values at concentration levels of 10, 60 and 100 mg kg⁻¹ amounted to 85 % for the ketchup and baby food matrices, 105 % for juices, 102 % for tomato purees, and 93 % for jams (9).

Incidence: The results revealed that 63% of the honey samples contained HMF concentrations higher than the permitted maximum limit of 40 mg kg⁻¹ as recommended by the Alimentarius Codex and ISIRI

(10, 12). These results are similar to those obtained by Makawi who reported that 18 out of the 28 honey samples from Sudan were contaminated with HMF levels ranging from 5 to 922 mg kg⁻¹ (23). The long-term storage is one of the critical factors affecting the amount of HMF in food; however, during the current study, the production date has not been considered. In a study conducted in Malaysia, HMF concentrations in fresh Malaysian honey samples, stored for at most six months, were within the internationally recommended range (2.80-24.87 mg kg⁻¹). While, the same honey samples when stored for 12 to 24 months contained much higher HMF concentrations ranging from 118.47 to 1139.95 mg kg⁻¹ (24).

The HMF concentrations in all of the jam samples (100%) were higher than 40 mg kg⁻¹ (the maximum acceptable level for honey). Our results were higher than those obtained by Mendoza and co-workers, who reported that HMF contents of the 38 jam samples analyzed varied from trace levels to 71.7 mg kg⁻¹ (2). The considerable variation in HMF content found in the analyzed jam samples may be due to differences in the processing, storage conditions, and natural fruit compositions (i.e. carbohydrate and sugar content, pH, and acidity). Storage, especially under improper conditions, is an important factor, leading to an increase in HMF levels. Mendoza declared that HMF values increased from 6 to 352 mg kg⁻¹ in stored commercial peach jams after a period of 12 months at 35°C (2). Similar results were observed in bitter and sweet orange jam samples by Kopjar (25).

The HMF concentration in tomato paste and ketchup showed significant differences, which may be contributed to the sugar and vinegar present in the ketchup recipes; the former increases the reducing sugar, and the latter decreases the pH. Ketchup is a high demanding condiment in the fast food industry, used as an accompaniment with various food items like French fries, burgers, chips, sandwiches, etc. The global ketchup market is growing rapidly along with increasing demand in children and adolescents (sensitive groups). Therefore, more attention is needed to control the HMF level in ketchup. Vorlova reported that, in 5 analyzed ketchup samples, HMF level ranged from 0.8–189.8 mg.kg⁻¹. They also mentioned that the total daily exposure to HMF from six investigated food commodity groups (jam, fruit, baby food, tomato puree, juice and syrup) for an average person in the Czech Republic may amount to 1.11 mg/person/day, of which the exposure to ketchups is the highest (up to 0.54 mg/person/day) (9).

The fruit juice samples also showed considerable amount of HMF. The measured values in the current study were higher than those reported by Mendoza (2) and Vorlova and colleagues (9), who obtained the average values of 2.9 and 7.2 mg kg⁻¹ for juices, respectively. In a related study, HMF level was determined in 20 samples of apple juice. In 17 (85%) samples, the concentration of 5-HMF was less than 20

mg kg⁻¹ and in 3 (15%) was higher than 20 mg kg⁻¹ (26).

Syrups samples in the present study showed higher concentrations of HMF compared to the canned fruit and fruit juice samples. These differences between fruit products were expected, since fruit juice and canned fruit are exposed to pasteurization temperatures or mild cooking, compared to the severe heating process used in manufacturing jams and fruit syrups. Higher temperatures result in severe Maillard reaction and caramelization. These reactions are also enhanced by the lower water activity values of jams and syrups. The influence of water activity and baking temperature on HMF formation in cookies was studied by Ameer and co-workers who stated that HMF formation depends on baking temperatures as well as water activity. Moreover, sugar content is higher in jams and syrups than in fruit juices and canned fruits (27).

The situation was different with regard to fruit cakes. The relatively high concentration of HMF in fruit cakes was expected. In a related study, Zhang and colleagues reported that sugar type, as well as baking temperature and time all had strong influences on HMF formation in sponge cakes. When the concentration of citric acid existing in the batter of sponge cakes increases, the conditions become more favorable for the formation of HMF (28).

We could not detect HMF in UHT milk, instant coffee and jelly powder samples. In contrast with our results, UHT milk and coffee are, generally, regarded as foods containing high amounts of HMF. Rehman and co-workers reported that HMF content in raw unheated milk was 7.66 µmol/litre, and in pasteurized and UHT milk, it ranged from 10.52-16.0 to 16.33-20.85 µmole/litre, respectively (29). In a related study in Spanish, HMF level in 24 commercial coffee substitutes and 12 instant coffees was ranged from 590 to 13000 mg kg⁻¹. Moreover, an estimation of the exposure of the Spanish population to HMF from heat-processed foods revealed that coffee and bread were responsible for nearly as high as 85% of the total daily HMF exposure levels, although UHT milk, biscuits, breakfast cereals, and tomato products were also important for their HMF exposure (13). The differences between our results with other researchers maybe related to limited number of samples (2

samples) in the current study. However, lower levels of HMF were found in the tomato paste, fruit juice, canned fruit and some fruit cake samples, but they are important sources adding to the HMF intake, especially since they are largely used by children. More research is needed to determine the total daily exposure levels to HMF from the consumption of different foods in Iran. The recovery values at HMF levels of 10, 60 and 100 mg kg⁻¹ amounted to 85 % for the ketchup and baby food matrices, 105 % for juices, 102 % for tomato purees, and 93 % for jams.

Conclusion: The results of our study suggest that, apart from honey, a number of other food commodities, including those containing fruit and/or sugar (e.g. jam, fruit cakes, and ketchup), hold considerable amounts of HMF. Regarding the fact that these foods are consumed mostly by children, monitoring HMF levels in foods seems to be necessary. Moreover, the extension of this work to include investigations from different parts of the world is essential to the accumulation of data about such products so as to be a guideline in setting maximum permitted levels of HMF in commonly available foods. Different kinds of jam must be analyzed to determine the factors that enhance HMF formation in them.

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References

1. Kowalski S, Lukasiwicz M, Duda-Chodak A, Ziec G. 5-Hydroxymethyl-2-Furfural (HMF) – Heat-induced formation, occurrence in food and biotransformation – a Review. *Pol., J Food Nutr Sci* 2013; 63: 207-225.
2. Mendoza RM, Olano A, Villamiel M. Determination of hydroxymethylfurfural in commercial jams and in fruit-based infant foods. *Food Chem* 2002; 79: 513–516.
3. Glatt H, Schneiderl H, Murkovic M, Monien BH, Meinel W. Hydroxymethyl-substituted furans: Mutagenicity in *Salmonella typhimurium* strains engineered for expression of various human and rodent sulphotransferases, *Mutagenesis* 2011; 1–8.
4. Belitz HD, Grosch W, Schieberle P. *Food Chemistry*, 4th ed. Berlin Heidelberg: Springer. 2009. p. 248–339.
5. Janzowski C, Glaab V, Samimi E, Schlatter J, Eisenbrand G. 5-hydroxymethylfurfural: Assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food Chem Toxicol* 2000; 38: 801-809.
6. Li Y, Lu X. Investigation on the origin of 5-HMF in Shengmai Yin decoction by RP-HPLC method. *Journal of Zhejiang University – Science B* 2005; 6: 1015–1021.
7. Durling LJK, Busk L, Hellman BE. Evaluation of the DNA damaging effect of the heat-induced food toxicant 5-hydroxymethylfurfural (HMF) in various cell lines with different activities of sulfotransferases. *Food Chem Toxicol* 2009; 47: 880–884.
8. Svendsen C, Husoy T, Glatt H, Paulsen JE, Alexander J. 5-Hydroxymethylfurfural and 5-Sulfoxymethylfurfural Increase Adenoma and Flat ACF Number in the Intestine of Min/+ Mice. *Anticancer Res* 2009; 29: 1921-1926.
9. Vorlova L, Borkovcova I, Kalabova K, Vecerek V. Hydroxymethylfurfural contents in foodstuffs determined by HPLC method. *J Food Nutr Res* 2006; 45: 34-38.
10. Codex Alimentarius, Revised codex standard for honey. *Codex Standard 12-1981, Rev. 1 (1987), Rev. 2. 2001.*
11. Council Directive 110/EC, Council Directive of 20 December relating to honey 2001/110/EC, Official Journal of the European Communities 2001.
12. Institute of Standards and Industrial Research of Iran, Honey- Specification and test methods. ISIRI no. 92. 7rd revision, Karaj: ISIRI; 2013 [in Persian].
13. Henares RJA, Cueva SP. Assessment of hydroxymethylfurfural intake in the Spanish diet, *Food Addit Contam* 2008; 25: 1306–1312.
14. Murkovic M, Pichler N. Analysis of 5-hydroxymethylfurfural in coffee, dried fruits and urine. *Mol Nutr Food Res* 2006; 50: 842–846.
15. Teixido E, Santos FJ, Puignou L, Galceran MT. Analysis of 5-hydroxymethylfurfural in foods by Gas Chromatography- Mass Spectrometry. *J Chromatogr A* 2006; 1135: 85–90.
16. Truzzi C, Annibaldi A, Illuminati S, Finale C, Rossetti M, Scarponi G. Determination of very low levels of 5-(hydroxymethyl)-2-furaldehyde (HMF) in natural honey: Comparison between the HPLC technique and the spectrophotometric white method. *J Food Sci.* 2012, 77: 784-790.

17. Zirbes L, Nguyen BK, Graaf D, Meulenaer B, Reybroeck W. Hydroxymethylfurfural: A possible emergent cause of honey bee mortality? *J Agric Food Chem* 2013; 49: 11865-70.
18. Windsor S, Kavazos K, Brooks P. The quantitation of hydroxymethylfurfural in Australian *Leptospermum* honeys. *J Pharmacognosy and Phytother* 2013; 5: 21-25.
19. Sevic R, Sen L, Nas S. Determination of colour quality and HMF content of unprocessed sultanas obtained from different vineyards. *IJRAFS* 2014; 2: 2311-2476.
20. Ferrer E, Alegria A, Courtois G, Farre R. Highperformance liquid chromatographic determination of Maillard compounds in store-brand and name-brand ultrahigh temperature-treated cows' milk. *J Chromatogr A* 2000; 881: 599-606.
21. Charlton AJ, Farrington WHH, Brereton P. Application of ¹H NMR and multivariate statistics for screening complex mixtures: Quality control and authenticity of instant coffee. *J Agric Food Chem.* 2002; 50: 3098-3103.
22. DIN, 10751-3, Analysis of honey: Determination of the content of hydroxymethylfurfural - Part 3: High performance liquid chromatographic method, 2000.
23. Makawi SZA, Taha MI, Zakaria BA, Siddig B, Mahmud H, Mohamed Elhussein AR. et al. Identification and Quantification of 5-Hydroxymethyl Furfural HMF in Some Sugar-Containing Food Products by HPLC, *Pakistan J Nutr* 2009; 8: 1391-1396.
24. Khalil MI, Sulaiman SA, Gan SH. High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year. *Food Chem Toxicol* 2010; 48: 2388-2392.
25. Kopjar M, Durkan I, Pilizota V. HMF formation and colour change of bitter orange and sweet orange jams during storage, *Croatian J Food Sci Technol* 2010; 2: 11-15.
26. Matic J, Saric B, Mandic AP, Milovnovic I, Jovanov P, Mastilovic J. Determination of 5-Hydroxymethylfurfural in apple juice. *Food Processing, Quality and Safety* 2009; 36: 35-39.
27. Ameer LA, Mathieu O, Lalanne V, Trystram G, Birlouez-Aragon I. Comparison of the effects of sucrose and hexose on furfural formation and browning in cookies baked at different temperatures. *Food Chem* 2007; 101: 1407-1416.
28. Zhang YY, Song Y, Hu XS, Liao XJ, Ni YY, Li QH. Effects of sugars in batter formula and baking conditions on 5-hydroxymethylfurfural and furfural formation in sponge cake models. *Food Reas Int* 2012; 49: 439-445.
29. Rehman ZU, Saeed A, Zafars SI. Hydroxymethylfurfural as an indicator for the detection of dried powder in liquid milk. *Milchwissenschaft* 2000; 55: 256 - 257.