Short communication: Determination of potential 5-hydroxymethyl-2-furaldehyde and 2-furaldehyde compounds in follow-on milks and infant formulas using the high-performance liquid chromatography method

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ABSTRACT

The aim of present study was to determine the levels of potential 5-hydroxymethyl-2-furaldehyde (HMF) and 2-furaldehyde (F) in 109 baby food samples (60 follow-on milks, 49 cereal- and milk-based infant formulas) obtained from different markets in Ankara (Turkey). Potential HMF and F compounds were determined by HPLC. Mean levels ($\pm$ standard error) of HMF and F of follow-on milk samples were found to be 237.85 ± 18.25 and 9.44 ± 0.39 $\mu\text{g}/100$ mL, respectively. Regarding the infant formulas, mean levels of HMF and F were found to be 905.41 ± 91.94 and 13.22 ± 1.21 $\mu\text{g}/100$ g. As a result, potential HMF was determined in all of the samples; potential F was determined in all the samples except 1. The mean levels of potential HMF and F of infant formulas were higher than mean levels of potential HMF and F of follow-on milks. In addition, HMF and F values of some samples with an imminent expiration date were found to be higher than HMF and F values of the other samples. At present, no limits have been established in the Turkish Food Codex (TFC) for furfural compounds concentrations in infant formula and milks. Establishing limits related to these compounds would be important for protecting the quality of infant foods.

Key words: follow-on milk, infant formula, 5-hydroxymethyl-2-furaldehyde, furfural

Short Communication

Milk and milk products are an important source of nutrients, such as animal protein and calcium, for humans, especially children (Pei et al., 2009; Er et al., 2010). Formation of toxic compounds affects the safety and nutritional value of foodstuffs (Friedman, 1996). Infant foods fulfill the nutritional requirements of the infants; however, composition of infant foods may

be affected by a Maillard reaction depending on raw material, infant formula composition, applied process, packaging, and storage conditions (Ferrer et al., 2000). A Maillard reaction occurs between amino groups and reduced sugars and affects the quality of foodstuffs (van Boekel, 1998). Infant foods are susceptible to Maillard reactions due to risk factors such as reduced sugar content, lysine-rich proteins, and high temperatures during production and storage time. Furthermore, when infant food is enriched with vitamin A, ferrous, and lactose, occurrence of Maillard reactions increases in comparison with milk (Chávez-Servín et al. 2005; Ferrer et al. 2005). Reduced sugars and lysine are responsible for early stages of the Maillard reaction, and in advanced stages of the reaction undesired compounds such as furfurals are formed (Ramírez-Jiménez et al., 2000; Ferrer et al., 2000). Potential 5-hydroxymethyl-2-furaldehyde or hydroxymethylfurfural (**HMF**) is defined as the sum of the precursor of HMF (HMF bound to protein, such as Amadori products or HMF from reduced sugars) and free HMF (Chávez-Servín et al., 2005). Many furfural compounds are formed in processed foods, such as sterilized or UHT milk, and dry foods at thermal treatment or storage under inappropriate temperatures. 5-Hydroxymethyl-2-furaldehyde, or hydroxymethylfurfural, is known as a main furfural compound and is related to browning reaction in some foods (Albalá-Hurtado et al., 1997); it also could be evaluated as a quality indicator of foods, including carbohydrates, during storage (Rada-Mendoza et al., 2002). 2-Furaldehyde, or furfural (**F**), is related to flavor changes in foodstuffs. Both HMF and F compounds are widely used as Maillard reaction indicators in many foodstuffs. The extent of the changes that occur due to the Maillard reaction is associated to the temperature-time parameter during the process (Albalá-Hurtado et al., 1997). Furfural compounds are specifically determined by strong UV absorption without formation of colored substances (Chávez-Servín et al., 2005). Interactions between infant food ingredients, especially a Maillard reaction, affect the carbohydrates and proteins. Furfural compounds, used to evaluate storage or thermal process, may occur in later stages

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of the Maillard reaction (Ferrer et al., 2002). Occurrence of HMF in food causes quality and nutritional deterioration. Therefore, determination and evaluation of this compound in foodstuffs are important. High-performance liquid chromatographic methods are used to determine furfural compounds accurately and reliably in foodstuffs (Morales and Jiménez-Pérez, 1998; 1999). The aim of the current study was to determine the HMF and F contents of follow-on milks and infant formulas consumed in Ankara, Turkey.

In the current study, 60 follow-on milks (liquid form) of 6 different brands (A, B, C, D, E, and F) and 49 infant formulas (powder form) of 3 different brands were used; infant formulas were milk-based (G, I, K) and cereal-based (H, J, L). The follow-on milk and infant formula samples had different serial numbers. Infant formula samples were packaged with PAP 21 (noncorrugated fiberboard) packaging materials (250 g). Follow-on milks were packaged with C/PAP 84 (paper and fiberboard/plastic/aluminum) packaging material (200 and 500 mL). The samples were collected from different markets in Ankara, Turkey. For analysis, HMF and F standards (Sigma-Aldrich, St. Louis, MO) were used. The oxalic acid ($C_2H_2O_4$) was purchased from Sigma-Aldrich (Steinheim, Germany), acetonitrile (CH_3CN) and TCA ($C_2HCl_3O_2$) were obtained from Merck Chemical (Darmstadt, Germany). All of the reagents were of HPLC or analytical grade. Deionized water was used throughout the experiments (Millipore Simplicity 185, Molsheim, France).

The extraction procedure for the analysis of HMF and F was based on the method described by Ferrer et al. (2005). For extraction of HMF and F from the baby food samples, 15 g of reconstituted infant formula (15%, wt/vol) or 10 g follow-on milk was transferred to a centrifuge tube, and 5 mL of 0.15 M oxalic acid (prepared daily) was added. The mixture was placed in a boiling water bath for 25 min (Memmert WB 10, Schwabach, Germany). After cooling at room temperature, 3 mL of a 40% (wt/vol) TCA solution was added and stirred thoroughly for 5 min. The mixture was centrifuged at $4,000 \times g$ for 15 min at room temperature (MSE, Mistral 1000, Loughborough, UK). The supernatant fluid was separated from the solid residue. The supernatant was collected and 10 mL of a 4% (wt/vol) TCA solution was added to the solid residue and stirred thoroughly for 10 min. After centrifugation ($4,000 \times g$, 15 min) at room temperature, both of the supernatants were transferred to a 50-mL flask. The final volume was measured and extract was filtered through a 0.20- μm filter (Millipore Corp., Bedford, MA) and then injected into the HPLC with diode array detector (DAD) system.

The analyses were performed using an HPLC apparatus (Agilent Series 1200, Santa Clara, CA) which

consisted of a DAD detector (Agilent G1314B VWD Series) set at 284 nm. The stationary phase was a Spherisorb (Waters, Dublin, Ireland) ODS2 (250×4.6 mm i.d., 5 μm) column. Chromatographic separation was performed at 25°C using a mixture of HPLC-grade water/acetonitrile (95:5 vol/vol) for the mobile phase at a flow rate of 1 mL/min. The calibration curve was determined using a series of dilutions containing different levels of HMF (0.05–10 $\mu g/mL$) and F (0.01–1 $\mu g/mL$). Injection volume was 20 μL for samples and standards.

The data were analyzed by one-way ANOVA. Significant differences of means were determined by the Duncan test (Daniel, 1991). All analyses were repeated 3 times for each sample.

Potential HMF and F were detected in the commercial follow-on milks and infant formula samples by using HPLC-DAD system. In the applied method, mean recoveries were 98.4% for HMF and 94.3% for F. Analytical parameters of the proposed HPLC method for potential HMF and F detection are shown in Table 1. The HMF levels were higher than F levels in all of the samples (Table 2). In commercial follow-on milk samples, mean levels ($\pm$ SE) of potential HMF and F were found to be 237.85 ± 18.25 and 9.44 ± 0.39 $\mu g/100$ mL, respectively. Minimum and maximum potential HMF and F levels of follow-on milks were determined to be 44.60 to 518.09 $\mu g/100$ mL and not detected to 14.18 $\mu g/100$ mL, respectively. Regarding the commercial infant formula samples, mean levels of potential HMF and F were found to be 905.41 ± 91.94 and 13.22 ± 1.21 $\mu g/100$ g. Minimum and maximum potential HMF and F levels of infant formulas were determined as 247.00 to 2,924.52 and 5.87 to 40.99 $\mu g/100$ g, respectively (Table 3). According to the statistical analysis for both HMF and F, the difference between brands was significant in follow-on milks ($P < 0.001$) and infant formulas ($P < 0.001$). Our data revealed that potential HMF was determined in all of the samples; potential F was determined in the all samples except 1. Some samples had higher HMF and F contents compared with others, likely because of the expiration dates of samples. These higher HMF and F values were maintained throughout the imminent expiration date of these samples.

Furfural contents of some foods have been determined in several studies (Burdurlu and Karadeniz, 2003; Tüfekci and Fenercioglu, 2010; Akpınar et al., 2011), but to our knowledge no study involving both HMF and F content in infant foods in Turkey has been reported. Furthermore, Gökmen and Şenyuva (2006) improved a method to determine HMF in baby foods. Those authors analyzed HMF in a wide variety of processed baby foods using a liquid chromatography-mass spectrometry method and found that HMF in

Table 1. Analytical parameters of the proposed HPLC method for 5-hydroxymethyl-2-furaldehyde (HMF) and 2-furaldehyde (F) detection

Parameter	HMF	F
Correlation of determination (R^2)	0.9998 (n = 9)	0.9991 (n = 7)
Regression equation	$y = 150.9x - 5.2689$	$y = 136.4x + 1.0715$
Limit of detection ($\mu\text{g/mL}$)	0.0048	0.0011
Limit of quantitation ($\mu\text{g/mL}$)	0.0159	0.0037
Repeatability		
Intraday (n = 5, RSD ¹ %)	1.08	1.52
Interday (5 d, n = 3, RSD %)	1.83	2.16

¹RSD = relative standard deviation.**Table 2.** Mean values of 5-hydroxymethyl-2-furaldehyde (HMF) and 2-furaldehyde of follow-on milks

Follow-on milk	n	Mean $\pm$ SE ($\mu\text{g}/100 \text{ mL}$)	Minimum ($\mu\text{g}/100 \text{ mL}$)	Maximum ($\mu\text{g}/100 \text{ mL}$)
HMF				
A	10	305.04 $\pm$ 11.89 ^b	258.93	395.20
B	10	82.84 $\pm$ 9.25 ^d	44.60	148.91
C	10	275.72 $\pm$ 7.69 ^b	246.92	323.27
D	10	459.43 $\pm$ 8.86 ^a	424.38	518.09
E	10	224.27 $\pm$ 33.83 ^c	65.09	318.82
F	10	79.80 $\pm$ 1.77 ^d	69.13	87.82
Average	60	237.85 $\pm$ 18.25	44.60	518.09
2-Furaldehyde				
A	10	10.57 $\pm$ 0.52 ^w	8.56	14.07
B	10	5.17 $\pm$ 0.28 ^z	ND ¹	7.06
C	10	12.04 $\pm$ 0.39 ^v	10.09	14.01
D	10	12.58 $\pm$ 0.22 ^v	11.66	14.18
E	10	9.08 $\pm$ 0.58 ^x	6.82	13.32
F	10	6.77 $\pm$ 0.46 ^y	5.43	10.66
Average	60	9.44 $\pm$ 0.39	ND	14.18

^{a-d}Means within a groups in a column (between HMF) not having a common superscript letter are different ($P < 0.001$).^{v-z}Means within a groups in a column (between F) not having a common superscript letter are different ($P < 0.001$).¹ND = not detected.**Table 3.** Mean values of 5-hydroxymethyl-2-furaldehyde (HMF) and 2-furaldehyde of infant formula

Infant formula	n	Mean $\pm$ SE ($\mu\text{g}/100 \text{ g}$)	Minimum ($\mu\text{g}/100 \text{ g}$)	Maximum ($\mu\text{g}/100 \text{ g}$)
HMF				
G	9	836.09 $\pm$ 84.05 ^{bc}	519.92	1,222.98
H	8	899.51 $\pm$ 39.32 ^{bc}	744.78	1,092.67
I	8	1,085.31 $\pm$ 80.25 ^b	783.97	1,464.72
J	8	1,636.67 $\pm$ 442.22 ^a	247.00	2,924.52
K	8	632.92 $\pm$ 73.86 ^{bc}	351.25	867.69
L	8	350.65 $\pm$ 24.51 ^c	280.48	487.97
Average	49	905.41 $\pm$ 91.94	247.00	2,924.52
2-Furaldehyde				
G	9	9.20 $\pm$ 0.87 ^z	5.87	13.11
H	8	16.51 $\pm$ 0.77 ^y	13.77	19.92
I	8	12.76 $\pm$ 1.59 ^{yz}	6.35	19.76
J	8	25.47 $\pm$ 4.81 ^x	12.48	40.99
K	8	6.75 $\pm$ 0.16 ^z	6.25	7.54
L	8	9.14 $\pm$ 0.59 ^z	7.45	12.46
Average	49	13.22 $\pm$ 1.21	5.87	40.99

^{a-c}Means within a groups in a column (between HMF) not having a common superscript letter are different ($P < 0.001$).^{x-z}Means within a groups in a column (between F) not having a common superscript letter are different ($P < 0.001$).

milk- and cereal-based foods ranged between 0.18 to 0.43 and 0.17 to 57.18 $\mu\text{g/g}$, respectively. Similar to the findings of the current study, those authors reported that cereal-based infant foods had higher HMF values against milk-based foods.

Several studies related to furfural compounds in infant foods have been conducted worldwide. The values of HMF and F in commercial liquid and powdered infant milk formulas were higher than those reported in commercial powdered cow milks in Spain (Albalá-Hurtado et al., 1997). Some authors have reported mean HMF and F contents of commercial liquid and powdered infant milk formulas and powdered cow milks to be 124.5 (38.3–175.2) and 16.0 (11.5–24.5), 111.5 (50.3–189.1) and 18.4 (16.2–22.9), and 50.7 (29.8–75.8) and 7.6 (4.3–10.1) $\mu\text{g}/100\text{ mL}$, respectively. Rada-Mendoza et al. (2002) analyzed HMF in 38 jams and 18 fruit-based infant foods in Spain and reported that fruit-based infant foods had lower mean HMF values (0.29 mg/100 g) compared with jam samples (1.35 mg/100 g). These HMF and F values in infant formulas were lower compared with the present study.

Likewise, furfural studies in stored infant foods have been performed by several researchers (Albalá-Hurtado et al., 1998, 1999; Ferrer et al., 2000, 2002, 2005; Guerra-Hernandez et al., 2002; Chávez-Servín et al., 2005, 2006). Albalá-Hurtado et al. (1998) investigated the changes of furfural compounds in powdered and liquid infant milks during 9 mo of storage at 20, 30, and 37°C in Spain and determined higher HMF and F content of powdered infant milk formula against liquid infant milk formula. Higher HMF and F contents in powdered infant formula were explained by the severe heat treatment at the production of the powdered infant milk formulas. Another study conducted by Albalá-Hurtado et al. (1999) also analyzed changes of furfural compounds in 3 different commercial infant milk formulas during 9 mo of storage at 20, 30, and 37°C in Spain. In that study, the authors reported that initial total HMF and F contents of 3.0 to 13.2 and 1.2 to 2.5 $\mu\text{mol/L}$, respectively. An increase in furfural compounds during the storage at 3 temperatures was determined, and heat treatment and composition of the milk was identified as an important factor that affected the furfural compounds. Ferrer et al. (2000) investigated the furfural compounds in infant foods and milk formulas by HPLC in Spain and determined that the HMF and F contents of infant foods to be 0.77 and 0.1 mg/100 g after 6 mo of storage, respectively. Guerra-Hernandez et al. (2002) reported that HMF and F were useful indicators to determine the stages of Maillard reaction and noted an increase in the HMF levels of the foods stored at 55°C when compared with 20°C. Ferrer et al. (2002) found an increase in HMF

and F amounts during storage and specified that storage temperature increases the total HMF concentration whereas storage time increases both total HMF and F concentrations. Ferrer et al. (2005) also stored the infant foods for 2 yr at 20 and 37°C and analyzed for furfural compounds such as 2-furaldehyde, 5-hydroxymethyl-2-furfuraldehyde, 2-furylmethylketone, and 5-methyl-2-furaldehyde by reversed phase-HPLC in Spain. Those authors determined a further increase in furfural compounds in infant foods samples stored at 37°C compared to samples stored at 20°C at the end of storage. Chávez-Servín et al. (2005) analyzed potential and free furfural compounds in milk-based formula for pregnant women in Spain and found the initial potential HMF value to be 902.81 $\mu\text{g}/100\text{ g}$; those authors also reported that after 15 mo of storage at 25 and 37°C, the HMF value increased to 1,562.30 and to 2,618.66 $\mu\text{g}/100\text{ g}$, respectively. Those authors determined initial potential F values to be 128.40 $\mu\text{g}/100\text{ g}$ and reported that after 15 mo of storage at 25 and 37°C it increased to 345.36 and to 515.96 $\mu\text{g}/100\text{ g}$, respectively. Chávez-Servín et al. (2006) also analyzed effect of long-chain PUFA on furfural compounds formation in the infant powder formula during the storage at 25 and 37°C in Spain. Those authors determined the initial potential HMF and F contents of supplemented and unsupplemented formula to be 485.88 and 167.13, and 515.85 and 170.29 $\mu\text{g}/100\text{ g}$, respectively. They concluded that the supplement with long-chain PUFA did not affect furfural compound formation.

Many factors, such as infant formula composition, processing, and storage, affect furfural compounds and affected our research. In the present study, HMF values of commercial baby foods according to type of product and brands were found to be different. The values of furfural compounds in the commercial infant foods cannot be compared because there is insufficient data reported in previous studies in Turkey. Infant foods are known as a fundamental food source in terms of nutrition for infants. For this reason, monitoring of deterioration products, such as furfural compounds, is important in infant foods. Establishing the limit for these compounds is important for protecting the quality of infant foods.

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