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# *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 (± standard error) of HMF and F of follow-on milk samples were found to be 237.85 ± 18.25 and 9.44 ± 0.39 μg/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 μg/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 × *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 × *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 μg/mL) and F (0.01–1 μg/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 (± SE) of potential HMF and F were found to be 237.85 ± 18.25 and 9.44 ± 0.39 μg/100 mL, respectively. Minimum and maximum potential HMF and F levels of follow-on milks were determined to be 44.60 to 518.09 μg/100 mL and not detected to 14.18 μg/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 μg/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 μg/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 Fenercioğlu, 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