Analytical Methods

Determination of furan and furan derivatives in baby food

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ABSTRACT

A Headspace-Solid Phase Microextraction–Gas Chromatography–Mass Spectrometry (HS-SPME–GC–MS) method was developed and validated for the simultaneous determination of furan, 2-methylfuran, 2-ethylfuran, 2-butylfuran, 2-pentylfuran, 2-acetylfuran, furfural and furfuryl alcohol in jarred baby food. The method was specific for the analytes. Good precision was obtained both in terms of intra-day repeatability (RSD ≤ 5.02) and inter-day precision (RSD ≤ 5.55%). The recovery values were between 98.42% and 99.8%. Linearity was established over two order of magnitude, and the achieved LODs and LOQs ranged between 0.018 and 0.035 ng/g and 0.060–0.117 ng/g, respectively, depending on the analyte. Finally, the method was successfully applied to investigate the content of furan and furan derivatives in several commercial baby food samples containing fruit or meat. The results showed that this simple, rapid and solvent free procedure could be used routinely for the analysis of baby food providing competent quantitative data functional to risk assessment.

1. Introduction

Furan and its derivatives are naturally occurring compounds formed in many heat-processed foods and drinks; these compounds have low odor thresholds and significantly contribute to the sensory properties of heated foods and beverages. Their presence in food was established in the 1960s as products or intermediates of the Maillard reaction (Grey & Shrimpton, 1967). Nevertheless, their mechanisms of formation remain unclear. It has been proposed that there are multiple precursors and alternative routes for the formation of furans in foods rather than a single mechanism; the major routes are the thermal degradation of carbohydrates and ascorbic acid and its derivatives and the thermal oxidation of polyunsaturated fatty acids (Crews & Castle, 2007). Perez Locas and Yaylayan (2004) suggested that ascorbic acid had the highest potential to produce furan, followed by some sugar/amino acids mixtures; reaction conditions, such as temperature, time and pH, can also affect the furan formation significantly (Fan, Huang, & Sokorai, 2008). In addition to thermal treatment, novel technologies are able to induce furan in food: furan levels up to 23.6 ng/mL and 60 ng/mL have been found in UV light treated apple cider (Fan & Geveke, 2007) and orange juice (Hu, Zhu, Hernandez, Koutchma, & Shao, 2016), respectively.

Recently, great attention has been paid to the presence of furan in foods by several international food organizations, such as the US Food and Drug Administration (US FDA) and the European Food Safety Authority (EFSA). Furan has been found to exhibit carcinogenic and cytotoxic activity on animals and harmful effects on human health (Byrns et al., 2006). It has been included in the Group 2B (possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC, 1995) and classified as a human pathogen (NTP, 2014) by the US Department of Health and Human Services.

Under these circumstances the US Food and Drug Administration, followed soon after by the European Food Safety Authority, began to collect information on the methods of analysis, occurrence and formation in food, exposure through consumption, and the toxicity of furan. In 2004 the FDA reported the first results on furan occurrence in a several selected foods, mainly heat-processed baby foods packaged in jars and cans (US FDA, 2004). In 2009, EFSA published the initial findings, updated in a 2010 report (EFSA, 2010), that summarized the data on furan concentrations in thousands food samples analyzed between 2004 and 2009 by various countries. Furan levels above 100 µg/

kg were found in coffee, baby food, sauces and soups.

Due to this increasing attention, in the last years analytical methods more and more selective and sensitive were developed and applied to the analysis of furan in food and baby food, mainly using HS-SPME coupled to GC–MS (Altaki, Santos, & Galceran, 2007; Bianchi, Careri, Mangia, & Musci, 2006; Goldmann, Périsset, Scanlan, & Stadler, 2005; Jestoi et al., 2009; Arisseto, Vicente, & Toledo, 2010; Kim, Kim, & Lee, 2010; Petisca, 2014; Pérez-Palacios, Petisca, Melo, & Ferreira, 2012; Ruiz, Santillana, Nieto, Cirugeda, & Sánchez, 2010).

In addition to furan, other furan derivatives can arise from the same routes of furan formation in food. These compounds, such as 2-alkyl-furans, 2-acetylfuran, furfural and furfuryl alcohol, have been re-cognized toxic for animals and humans and a concern for their geno-toxicity in humans was identified by EFSA in 2011 (EFSA, 2011; Okaru & Lachenmeier, 2017; Ravindranath, McMenamin, Dees, & Boyd, 1986; Sujatha, 2008). More recently, furfuryl alcohol has been subjected to a risk assessment by the International Agency for Research in Cancer (IARC, 2017). Considering that many furan derivatives are also added to foodstuffs as flavoring substances, data on their naturally occurring amount in food could be useful to assess the implications for human health. Conversely, to date the information on the occurrence of furan derivatives in foods and beverages and even in baby foods are limited; the available information only regard the content of furfural and hy-droxymethylfurfural (HMF) in fruit-based baby foods (Mesias-Garcia, Guerra-Hernández, & García-Villanova, 2010; Čížková, ŠevČík, Rajch, & Voldřich, 2009), 2- and 3-methylfuran in foods and baby foods (Becalski et al., 2010), furfural, furfuryl alcohol, 2-penthylfuran in coated deep-fried products (Petisca, 2014; Pérez-Palacios et al., 2012), 2-methylfuran and 2-penthylfuran in fruit juice (Hu et al., 2016) and finally 2-alkylfurans (2-methylfuran, 2-ethylfuran, 2,5-dimethylfuran, 2-propylfuran, 2-butylfuran, 2-pentylfuran) in a large variety of food-stuffs including baby foods (Shen et al., 2016).

In this context, the aim of this research was to develop and validate a simple and rapid HS-SPME-GC–MS method for the simultaneous quantification of furan and several alkylated and oxygenated furan derivatives, namely 2-methylfuran, 2-ethylfuran, 2-butylfuran, 2-pen-tylfuran, 2-acetylfuran, furfural and furfuryl alcohol, in baby food.

2. Materials and methods

2.1. Samples collection

24 different baby food samples, belonging to the major brands available on the Italian market, were purchased from local retailers in October 2014. Two different product categories were represented, namely fruit- and meat-based purées packed in glass-jars. Exactly, eight fruit-based samples, specifically apple (1 sample), apple-banana (2 samples), apple-apricot (1 sample), pear (3 samples) and multifruits (1 sample), and sixteen meat-based samples, namely veal (4 samples), beef (4 samples), chicken (4 samples) and turkey (4 samples), were col-lected. Their labeled ingredients and percentage composition were re-ported in Table 1. The sample jars were stored unopened at +4 °C until the analyses that were performed in triplicate by nine months from their purchasing and, in any case, before their expiration date.

2.2. Chemicals and reagents

Furan (≥99%), 2-methylfuran (≥99%), 2-ethylfuran (≥99%), 2-butylfuran, 2-pentylfuran (> 98%), 2-acetylfuran (> 99%), furfuryl alcohol (≥98%), furfural (99%), acetone (capillary GC grade, ≥99.9%), water (Chromasolv®, for HPLC), *n*-alkane mixture (C_6-C_{28} in hexane) and sodium chloride (≥99.0%) were supplied by Sigma-Aldrich (Milan, Italy).

2.3. Standard solutions

Standard stock solutions of furan (1.05 mg/mL), 2-methylfuran (0.12 mg/mL), 2-ethylfuran (0.19 mg/mL), 2-butylfuran (0.10 mg/mL), 2-pentylfuran (0.15 mg/mL), 2-acetylfuran (0.13 mg/mL), furfural (1.03 mg/mL) and furfuryl alcohol (1.01 mg/mL) in acetone were pre-pared and stored at 0 °C. Calibration curves were constructed using a set of mixtures of furan and furan derivatives in water of analytical grade; the mixtures were prepared by diluting different volumes of the stock solutions in HPLC-grade water completed to 10.0 mL final volume. The mixtures of standard stock solutions were prepared daily and stored at +4 °C.

2.4. Headspace-Solid Phase Microextraction (HS-SPME) procedure

For the extraction of furan and furan derivatives the Headspace-Solid Phase Microextraction technique was applied under the optimized extraction conditions. In particular, a 40 mL vial was filled with about 10 g, exactly weighed, of each sample previously refrigerated, and 10 mL of saturated aqueous NaCl solution. The vial was equipped with a "mininert" valve (Supelco, Bellefonte, PA, USA) that allowed the in-troduction of the fiber without piercing any septum avoiding extra-neous peaks due to possible septum bleeding. Extraction was performed in the headspace vial kept at 35 °C using a divinylbenzene/carboxen/polysimethylsiloxane (DVB/CAR/PDMS) fiber of 50/30 µm film thick-ness (Supelco, Bellefonte, PA, USA), housed in its manual holder (Supelco, Bellefonte, PA, USA). The fiber was conditioned according to the manufacturer's instructions prior to use.

The sample was equilibrated for 15 min and then extracted for 15 min. During the extraction, the sample was continuously stirred. After the sampling, the SPME fiber was introduced onto the splitless injector of the GC–MS, maintained at 260 °C, and there kept for 3 min for thermal desorption of the analytes onto the capillary GC column. No artifacts were observed after a SPME analysis of the saturated saline solution performed as blank analysis.

To optimize the technique, the performance of CAR/PDMS vs DVB/CAR/PDMS fiber was tested and the effects of several variables, such as sample/saline solution ratio, extraction time and temperature and desorption time and temperature, on the extraction efficiency were studied. The criteria used as measure of efficiency were the desorption peak area (total ion chromatogram) and the coefficient of variation (CV %) of the measurements.

2.5. Gas Chromatograph–Mass spectrometer (GC–MS) analysis

A Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Turin, Italy) was used for GC–MS analysis of the volatile compounds. Helium was used as the carrier gas at a constant pressure of 10 psi; the gas chromatograph was operated in splitless mode with injector maintained at the temperature of 260 °C. Chromatographic separation was performed on a CP-Wax 52 CB, 60 m, 0.25 mm i.d., 0.25 µm film thickness (Chrompack Italy, s.r.l. Turin, Italy). The following GC oven temperature program was applied: 35 °C held for 5 min, then increased to 80 °C at a rate of 3 °C/min and to 250 °C at 10 °C/min, held at 250 °C for 15 min. Transfer line was maintained at the temperature of 250 °C. The mass spectrometer was operated in total ion monitoring mode with an acquisition range of 40–200 *m/z*. Each compound was identified using mass spectral data, NIST 14 library (NIST/EPA/NIH Mass Spectra Library, version 2.0 g, USA), linear retention indices (LRI), literature data and the injection of standards (Cincotta, Verzera, Tripodi, & Condurso, 2017).

2.6. Quantitative analysis

Furan and furan derivatives were quantified by using the method of standard additions. Mixtures of standard solutions containing furan and

Table 1
Labeled ingredients and nutritional value (g/100 g) of the analyzed jarred baby food samples.

Brand	Sample	Ingredients	Total/Saturated fat	Total Carbohydrate/Sugar	Protein (N × 6.25)	Salt	Vitamin C
A	Apple-Banana	Banana (50%), apple (49.9%), concentrated lemon juice, vitamin C.	0.2/0	13.4/12.2	0.6	0.002	0.028
A	Multifruits	Banana (42%), pear (38%), apple (15%), concentrated orange juice (2.5%), concentrated lemon juice, vitamin C.	0.3/0.1	17.0/14.0	0.4	0.002	0.028
A	Veal	Water, veal (40%), rice starch, corn starch, vegetable oil.	4.0/ < 1.5	4.2/1.0	7.7	0.093	–
A	Beef	Water, beef (40%), rice starch, corn starch, vegetable oil.	3.8/1.4	4.5/0.5	7.6	0.03	–
A	Chicken	Water, chicken (40%), rice starch, corn starch, vegetable oil.	3.6/1.2	4.0/1.0	7.0	0.083	–
A	Turkey	Water, turkey (40%), rice starch, corn starch, vegetable oil.	3.5/0.95	4.5/0	7.2	0.088	–
B	Apple	Apple (60%), water, sugar, corn starch, concentrated lemon juice, vitamin C.	0.2/0.1	16.0/13.0	0.2	0.004	0.050
B	Pear	Pear (60%), water, sugar, corn starch, concentrated lemon juice, vitamin C.	0.2/0	16.0/13.0	0.3	0.004	0.050
B	Veal	Water, veal (30%), corn starch, rice flour, salt, concentrated lemon juice.	3.0/1.4	7.9/0.1	5.1	0.05	–
B	Beef	Water, beef (30%), corn starch, rice flour, salt, concentrated lemon juice.	3.0/1.4	7.4/0.1	5.1	0.05	–
B	Chicken	Water, chicken (30%), corn starch, rice flour, salt, concentrated lemon juice.	2.5/0.5	5.9/0.1	5.6	0.05	–
B	Turkey	Water, turkey (30%), corn starch, rice flour, salt, concentrated lemon juice.	2.5/0.5	7.2/0.1	5.3	0.05	–
C	Apple-Banana	Apple (45%), banana (22%), water, orange juice, corn starch, sugar, lemon juice, vitamin C.	0.1/0	11.8/8.8	0.4	< 0.03	0.030
C	Pear	Pear (88,4%), water, corn starch, sugar, vitamin C.	0.2/0	15/14	0.2	< 0.03	0.030
C	Veal	Water, veal (40%), corn starch, sunflower oil, salt.	3.1/0.4	6.7/0.01	5.7	0.1	–
C	Beef	Water, beef (40%), rice starch, corn starch, salt, sunflower oil.	3.7/1.3	6.6/0.01	6.2	0.1	–
C	Chicken	Water, chicken (40%), rice starch, corn starch, sunflower oil, salt.	2.0/0.4	6.4/tr	6.4	0.1	–
C	Turkey	Water, turkey (30%), corn starch, rice flour, rice starch, sunflower oil.	2.2/0.5	6.5/tr	6.0	0.1	–
D	Apple-Apricot	Apple (63%), apricot (30%), corn starch, concentrated lemon juice, vitamin C.	0.4/0.2	14.0/10.0	0.4	0.01	0.050
D	Pear	Pear (96%), corn starch, concentrated lemon juice, vitamin C.	0.2/0.1	17.0/11.4	0.3	0.01	0.050
D	Veal	Water, veal (40%), corn starch, sunflower oil, salt, concentrated lemon juice.	3.6/1.7	6.9/0.1	6.0	0.08	–
D	Beef	Water, beef (40%), corn starch, sunflower oil, salt, concentrated lemon juice.	3.6/1.7	7.4/0.1	6.0	0.08	–
D	Chicken	Water, chicken (30%), rice flour, corn starch, salt, concentrated lemon juice.	2.5/0.6	6.5/0.1	6.4	0.08	–
D	Turkey	Water, turkey (30%), corn starch, rice flour, concentrated lemon juice.	2.5/0.6	6.5/0.1	6.2	0.08	–

furan derivatives standards in a ratio 0.6–1.4 times those of the corresponding analytes were added to multiple aliquots of each sample. The sample alone was also analyzed. Quantitation was based on a seven point calibration curve generated by plotting detector response versus the amount spiked of each standard. Each sample measurement was repeated three times. The samples were extracted and analyzed by HS-SPME–GC–MS as previously described.

2.7. Validation

The following validation parameters were determined: specificity, precision, accuracy, linearity, limit of detection (LOD) and limit of quantification (LOQ).

Specificity was tested verifying the lack of detector response at the retention time of the analytes by analyzing in triplicate 3 blank samples (homemade apple purées). A response was considered to be present if it was ≥ the value of the detection limit.

Intraday and interday precision were calculated using the analyzed samples. For intraday precision, samples were analyzed five times consecutively and for interday precision, samples were analyzed twelve times in three nonconsecutive days. Precision was expressed as the relative standard deviation (RSD %).

The accuracy of the method was evaluated through recovery studies that were performed by spiking blank sample (homemade apple purée) with 10 ng/g of each furan derivative; the spiked sample was analyzed three times. The accuracy was expressed as percentage of recovery.

The method linearity was evaluated using a set of mixtures of furan and furan derivatives in water of analytical grade added to blank samples (homemade apple purées).

Limits of quantification (LOQ) and detection (LOD) were calculated from specific calibration curves constructed using blank samples containing the analytes in the range of their limits of detection. The slope (m) and the standard deviation of y-intercept (σ) of the regression lines were thus used for LOD and LOQ calculation using Eqs. (1) and (2), respectively

$$LOD = 3 \times \sigma/m \tag{1}$$

$$LOQ = 10 \times \sigma/m \tag{2}$$

where σ is the standard deviation of the intercept and m is the slope of the calibration curve.

2.8. Statistical analysis

Statgraphics Plus Version 5.1 software was used to perform statistical analysis of the data. One-way analysis of variance (ANOVA) and Duncan's multiple range test were applied to the data to determine significant differences among the analyzed samples.

3. Results and discussion

3.1. HS-SPME-GC–MS optimization

Firstly, the gas-chromatographic parameters for the optimization of the HS-SPME-GC–MS method were set. The stationary phase of capillary column, the programming oven temperature and the flow of carrier gas were investigated for the optimal separation and response of furan and furan derivatives using mixed standard solutions. The use of a 60 m polar column with an oven temperature starting from 35 °C and slowly ramping to 250 °C allowed a good separation of all the analytes without broadening of later eluting peaks (Fig. 1). The splitless mode was chosen to ensure the complete transfer of analytes and to increase the sensitivity.

For the HS-SPME extraction a DVB/CAR/PDMS fiber was used. Some Authors (Bianchi et al., 2006; Goldmann et al., 2005) have assessed the performance of different fibers in the extraction of furan

from foods and baby foods verifying that CAR/PDMS fiber showed the best sensitivity. Since then, CAR/PDMS fiber has been largely used for the furan extraction from foods and beverages (Hu et al., 2016; Petisca, 2014; Pérez-Palacios et al., 2012). In this research the performance of CAR/PDMS vs DVB/CAR/PDMS fiber was tested and it resulted that for the simultaneous extraction of furan, alkylated furans and oxygenated furan derivatives, such as furfural, furfuryl alcohol and 2-acetylfuran, the DVB/CAR/PDMS showed the best performance.

To establish the optimum conditions for extraction, several variables, such as sample/saline solution ratio, sample heating temperature, extraction time, desorption time and temperature, were considered.

Each sample was suspended in a saline solution (saturated aqueous solution of NaCl) to favor the magnetic stirring which plays an important role in increasing the SPME reproducibility and shortening the equilibrium time. As already demonstrated in our previous works (Verzera, Condurso, Romeo, Tripodi, & Ziino, 2010; Verzera, Ziino, Condurso, Romeo, & Zappalà, 2004), the use of a saturated aqueous NaCl solution improves the extraction efficiency of the analytes since it decreases the solubility of organic compounds and thereby increase the distribution constant between the sample matrix and the fiber coating sorbent. Different amounts of baby food and saline solution were tested; 10 g of baby food and 10 mL of saline solution with a sample/headspace volume ratio of about 1:1 provided the highest peak areas for most of the extracted compounds.

Extraction temperature is an important factor affecting the adsorption of analytes and thereby the extraction efficiency. In the present study, different sample heating temperatures were tested, namely 25, 30 and 35 °C. An evident increase of the peak areas of all analytes was observed from 25 °C to 30 °C and up to 35 °C. Trials were not carried out at higher temperatures since it has been demonstrated (Bicchi et al., 2011) that furan formation occurs in samples heated at temperatures greater than or equal to 40 °C. Therefore, an extraction temperature of 35 °C was selected for all the subsequent experiments.

Extraction time is another parameter carefully assessed in the development of this SPME method. Appropriate time is needed to achieve distribution equilibrium of the analytes among the headspace phase, the solution matrix and the fiber coating. A sampling time of 15 min proved to be optimal for most of the compounds of interest. The sampling step was preceded by an equilibrium time of 15 min; the sample was preheated so that the thermal equilibrium had reached when the fiber was introduced into the headspace.

The injector temperature recommended by the manufacturer for the DVB/CAR/PDMS fiber ranges between 230 and 270 °C; the desorption temperature of 260 °C was able to fully desorb the compounds from the fiber in 3 min; no carryover was observed in the following blank injection.

Based on the above optimized SPME parameters, the optimal conditions for SPME procedure were reported in the Section 2.4 (Headspace-Solid Phase Microextraction (HS-SPME) procedure).

3.2. Method validation

The analytical performance of the HS-SPME-GC–MS method for reliable quantification of furan, 2-methylfuran, 2-ethylfuran, 2-butylfuran, 2-pentylfuran, 2-acetylfuran, furfural and furfuryl alcohol in baby foods was evaluated. The method was validated in terms of detection limit, quantitation limit, linearity, precision and accuracy by using the experimental setting providing the optimized conditions. Quality parameters of the method are summarized in Table 2.

The specificity of the method was determined by analyzing in triplicate 3 blank samples (homemade apple purées); from the analysis no signals resulted at the retention time of the analytes (furan, 2-methylfuran, 2-ethylfuran, 2-butylfuran, 2-pentylfuran, 2-acetylfuran, furfural and furfuryl alcohol), thus the method can be considered specific for the analytes.
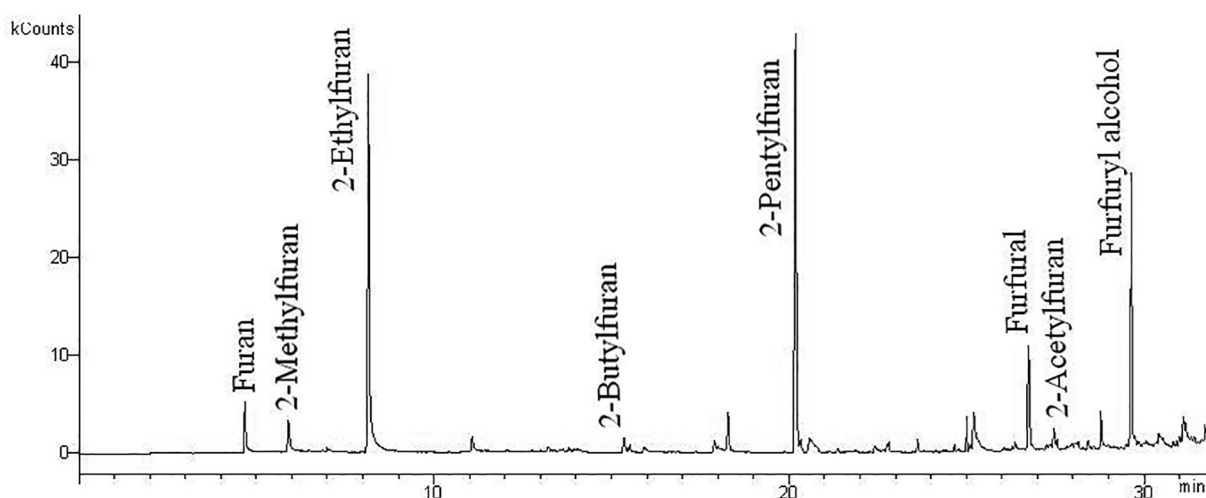
Fig. 1. HS-SPME-GC–MS chromatogram (SIM: 68 *m/z*, 81 *m/z*, 95 *m/z*) of furan and furan derivatives in a meat-based baby food sample analyzed.

The accuracy of the developed method was assessed calculating the percentage of recovery of each analyte from a blank sample spiked with 10 ng/g of each compound. The extraction recovery varied between 98.42% and 99.8% depending on the compound, the lowest value being found for furfural and furfuryl alcohol. The results are listed in Table 2.

Method precision was evaluated by replicate injections of the same samples. Good results were obtained both in terms of intra-day repeatability and inter-day precision: RSD% values between 2.35% and 5.02% were obtained for intra-day repeatability and between 3.89% and 5.55% for inter-day precision (Table 2). These data indicated that the method was highly accurate and reproducible.

The developed method showed an excellent linear relationship over two order of magnitude between the peak area and the concentration for all the compounds with correlation coefficients (r^2) higher than 0.999 and linear ranges 1.05–245.80 ng/g for furan, 0.12–25.00 ng/g for 2-methylfuran, 0.19–25.30 ng/g for 2-ethylfuran, 0.10–24.90 ng/g for 2-butylfuran, 0.15–25.10 ng/g for 2-pentylfuran, 0.13–24.80 ng/g for 2-acetylfuran, 1.03–238.20 ng/g for furfural, 1.01–240.60 ng/g for furfuryl alcohol (Table 2).

LOD and LOQ values, expressed as concentration (ng/g of food), were calculated from the calibration curve of each analyte using the slope and the standard deviation of the intercept. Excellent results were obtained; in particular, the achieved LODs and LOQs were 0.029 ng/g and 0.097 ng/g for furan, 0.031 ng/g and 0.103 ng/g for 2-methylfuran, 0.028 ng/g and 0.093 ng/g for 2-ethylfuran, 0.019 ng/g and 0.095 ng/g for 2-butylfuran, 0.018 ng/g and 0.090 ng/g for 2-pentylfuran, 0.034 ng/g and 0.113 ng/g for 2-acetylfuran, 0.033 ng/g and 0.110 ng/g for furfural, 0.035 ng/g and 0.117 ng/g for furfuryl alcohol, respectively (Table 2).

LOD and LOQ values of furan were similar to those described by Bianchi et al. (2006) that reported 25.7 ng/kg and 41.7 ng/kg by SPME-

GC–MS, respectively, but lower than those described by other authors for foods and baby foods (Arisseto et al., 2010; Hu et al., 2016; Jestoi et al., 2009; Kim et al., 2010; Pérez-Palacios et al., 2012).

Regarding the LOD and LOQ values of the furan derivatives, a comparison with literature data can be done only for 2-methylfuran, 2-pentylfuran, furfural and furfuryl alcohol since the others have never been determined by SPME-GC–MS. Hu et al. (2016) quantified 2-methylfuran and 2-pentylfuran in fruit juice with detection limits of 0.042 ng/mL and 0.23 ng/mL respectively, whereas Pérez-Palacios et al. (2012) achieved detection limits of 0.06 µg/g, 0.01 µg/g and 1.50 µg/g for 2-pentylfuran, furfural and furfuryl alcohol, respectively, in coated deep-fried products. The detection limits of these compounds in the present study were very close to those reported earlier for 2-methylfuran and more sensitive for 2-pentylfuran, furfural and furfuryl alcohol.

3.3. Furan and furan derivatives in baby food

The developed method was applied to quantify furan, 2-methylfuran, 2-ethylfuran, 2-butylfuran, 2-pentylfuran, 2-acetylfuran, furfural and furfuryl alcohol in the jarred baby food samples (Table 3). Furan was detected in all the samples at concentration levels ranging from 3.78 ng/g to 31.04 ng/g. The analysis of variance revealed that the fruit-based baby foods showed the lowest furan concentrations (3.78–4.16 ng/g): no statistically significant differences were observed among fruit-based samples depending on brand or kind of fruit. An opposite trend resulted for the meat-based samples that presented furan levels ranging between 18.53 ng/g and 31.04 ng/g with statistically significant differences among samples from different brand and/or containing different kinds of meat. In particular, the highest furan levels resulted for veal and beef samples from brands A, B and C and for

Table 2
Quality parameter of furan and furan derivatives detected under the optimized HS-SPME-GC–MS method.

Compounds	Calibration curve /Method linearity			Precision		Recovery (%)	LOD (ng/g)	LOQ (ng/g)
	Linear range (ng/g)	Slope	R^2	Intraday RSD%	Interday RSD%			
Furan	1.05–245.80	2.812	0.9996	2.35	3.89	99.8	0.029	0.097
2-Methylfuran	0.12–25.00	2.304	0.9998	3.24	4.05	99.7	0.031	0.103
2-Ethylfuran	0.19–25.30	2.782	0.9996	4.41	4.99	99.6	0.028	0.093
2-Butylfuran	0.10–24.90	1.925	0.9998	4.22	4.87	99.0	0.019	0.063
2-Pentylfuran	0.15–25.10	2.018	0.9995	3.58	4.12	99.1	0.018	0.060
2-Acetylfuran	0.13–24.80	1.889	0.9997	5.02	5.24	98.9	0.034	0.113
Furfural	1.03–238.20	1.993	0.9997	5.01	5.55	98.4	0.033	0.110
Furfuryl alcohol	1.01–240.60	1.896	0.9997	4.98	5.18	98.5	0.035	0.117

Table 3
Furan and furan derivatives concentration (ng/g) in the analyzed jarred baby food samples.

Brand	Product	Furan	2-Methylfuran	2-Ethylfuran	2-Butylfuran	2-Pentylfuran	2-Acetylfuran	Furfural	Furfuryl alcohol
A	Apple-Banana	3.89 a[*]	0.31 a	0.36 a	tr[†] a	4.93 a	-[‡] a	8.06 a	– a
B	Apple	3.95 a	0.30 a	0.35 a	tr a	4.89 a	- a	8.09 a	– a
C	Apple-Banana	3.78 a	0.32 a	0.36 a	tr a	4.95 a	- a	8.01 a	– a
D	Apple-Apricot	4.16 a	0.32 a	0.37 a	0.064b	5.01 a	0.115c	8.11 a	1.25c
A	Multifruits	4.12 a	0.33 a	0.36 a	0.065b	5.04 a	0.121c	8.16 a	1.28c
B	Pear	4.02 a	0.32 a	0.37 a	tr a	4.98 a	tr b	8.21 a	tr b
C	Pear	4.19 a	0.33 a	0.37 a	tr a	4.92 a	tr b	8.19 a	tr b
D	Pear	4.03 a	0.33 a	0.36 a	0.064b	5.02 a	tr b	8.17 a	tr b
A	Veal	30.14 d	3.52 d	11.23 d	1.46 e	14.01 d	5.11f	28.01 d	20.48f
B	Veal	29.94 d	3.48 d	11.31 d	1.55 e	13.89 d	5.02f	27.98 d	20.37f
C	Veal	31.04 d	3.59 d	11.19 d	1.51 e	13.75 d	5.25f	27.35 d	21.35f
D	Veal	24.05c	3.01c	9.01c	1.29 d	10.25c	4.81 e	25.11c	16.89 e
A	Beef	28.91 d	3.47 d	11.21 d	1.49 e	13.81 d	5.21f	27.59 d	21.03f
B	Beef	30.39 d	3.53 d	11.29 d	1.57 e	13.95 d	5.29f	27.91 d	21.14f
C	Beef	29.15 d	3.48 d	11.25 d	1.58 e	13.59 d	5.28f	27.37 d	20.42f
D	Beef	23.92c	2.99c	9.11c	1.31 d	10.35c	4.91 e	24.53c	15.91 e
A	Chicken	29.45 d	3.51 d	11.28 d	1.58 e	14.03 d	5.18f	27.86 d	20.82f
B	Chicken	23.96c	3.02c	9.19c	1.31 d	10.29 d	4.79 e	25.02c	16.58 e
C	Chicken	19.19b	2.24b	7.86b	0.99c	7.85b	2.51 d	20.31b	9.86 d
D	Chicken	23.54c	3.01c	9.51c	1.28 d	10.31c	4.82 e	24.87c	16.47 e
A	Turkey	30.09 d	3.49 d	11.30 d	1.52 e	14.01 d	5.19f	27.02 d	20.79f
B	Turkey	24.59c	3.03c	9.42c	1.29 d	10.27c	4.95 e	24.93c	16.51 e
C	Turkey	18.53b	2.19b	7.91b	0.91c	7.91b	2.35 d	20.34b	10.03 d
D	Turkey	23.89c	3.02c	9.48c	1.29 d	10.35c	4.86 e	25.31c	16.51 e

[*] Different letters in the same column indicate significant differences at P < .05 by Duncan's multiple range test.
[†] Not detected.
[‡] < to LOQ.

chicken and turkey from brand A, whereas the lowest for chicken and turkey samples from brand C.

The presence of furan in all the samples is due to the multiple routes of its formation from different precursors, such as carbohydrates, amino acids, lipids, and ascorbic acid and its derivative dehydroascorbic acid. In agreement with the findings of Limacher, Kerler, Davidek, Schmalzried, and Blank (2008) and Fan et al. (2008), the most potential precursors for furan in baby foods seem to be polyunsaturated lipids such as polyunsaturated fatty acids and carotenoids (Becalski & Seaman, 2005; Perez Locas & Yaylayan, 2004), whereas furan formation from sugars and ascorbic acid represents a minor route. Looking at Table 1, it is possible to see that the furan amount increased as unsaturated fat content increased, thus the lipid composition of baby food seems to be a key factor in furan formation.

Furan concentration in the analyzed baby food samples was in the range of that reported in the EFSA (2010) and US FDA (2004) database and in other studies (Becalski et al., 2005; Altaki et al., 2007; Bianchi et al., 2006; Ruiz et al., 2010; Zoller, Sager, & Reinhard, 2007). In particular, the amount of furan reported in the literature for fruit-based baby food is in most case lower than 8 ng/g as it resulted in our samples. Regarding the meat-based baby food, levels of furan up to 90 ng/g were reported in the US FDA (2004) database and by Jestoi et al. (2009), whereas up to 135 ng/g by Zoller et al. (2007). Furan amounts ranging between 10.7 ng/g and 95.5 ng/g were reported in Brazilian meat-based baby food samples (Arisseto et al., 2010) whereas furan levels more similar to those determined in our samples were found in meat-based baby food from the Spanish market (Ruiz et al., 2010).

The fruit-based jarred baby food samples showed significantly lower concentrations when compared to meat-based samples with regard to the furan derivatives, too. In particular, 2-butylfuran was detected in trace or at very low concentrations (0.064–0.065 ng/g) in all the fruit samples analyzed, 2-methylfuran and 2-ethylfuran were always below 0.4 ng/g, whereas 2-pentylfuran amounts (4.89–5.04 ng/g) resulted similar to those of furan; no statistically significant differences were observed for the 2-alkylfurans among the analyzed fruit samples, except for 2-butylfuran. Among oxygenated furan derivatives only furfural was detected in all the fruit samples, with no statistical differences among

the samples; its content (8.01 and 8.21 ng/g) exceeded the levels of furan and other furan derivatives. 2-Acetylfuran and furfuryl alcohol were the less represented in the fruit-based samples: they were detected only in two samples at concentration levels of about 0.1 ng/g and 1 ng/g, respectively.

Additionally, the content of furan derivatives resulted statistically significant different among the meat-based jarred baby food samples. As reported in Table 3, the amounts of furan derivatives increased as furan content increased. In meat samples, 2-butylfuran (0.91 ng/g and 1.58 ng/g) was the less represented followed, in increased order of concentration, by 2-methylfuran (2.19–3.59 ng/g), 2-acetylfuran (2.35–5.29 ng/g), 2-ethylfuran (7.86–11.31 ng/g), 2-pentylfuran (14.01–7.85 ng/g), furfuryl alcohol (9.86–21.35 ng/g) and furfural (20.31–28.01 ng/g); none of these compounds exceeded the levels of furan (18.53 ng/g–31.04 ng/g).

2-Akylfurans mainly resulted from lipid degradation as it probably happens for furan. In fact, it has been proved that 2-alkylfurans are oxidation products of polyunsaturated fatty acids (Adams, Bouckaert, Van Lancker, De Meulenaer & De Kimpe, 2001; Perez Locas & Yaylayan, 2004) and, in particular, 2-pentylfuran of linoleic acid (Min, Callison, & Lee, 2003). 2-Methylfuran can arise also from sugar solution by thermal treatment but to a lesser extent (Limacher et al., 2008). Furfural is formed in processed food from ascorbic acid during thermal treatment or storage but also from the degradation of sugars (Yuan & Chen, 1998); its formation via non-enzymatic browning reaction is often used as the indicator of time-temperature effort within the production and storage.

Little has been reported in the literature on the content of these furan derivatives in jarred baby food. Becalski et al. (2010) quantified 2-methylfuran in 17 commercial baby foods and reported concentration levels between 1.18 ng/g and 50.2 ng/g with the lowest levels being found in fruit-based products whereas Shen et al. (2016) reported up to 13.5 ng/g, 279.4 ng/g, 4 ng/g and 229.7 ng/g for 2-methylfuran, 2-ethylfuran, 2-butylfuran and 2-pentylfuran, respectively in baby food purees. Čížková et al. (2009) observed an average furfural content of 253 μg/100 g in fruit-based baby food, whereas values up to 182 μg/100 g have been reported by Mesias-Garcia et al. (2010).

It is noteworthy that, due to the multiple precursors and alternative

routes of formation, the amount of furan and furan derivatives in food can be affected by several factors such as food composition, pH, heating time and temperature during processing, time of storage etc. (Becalski et al., 2010; Fan et al., 2008; Perez Locas & Yaylayan, 2004). Moreover due the high volatility of these compounds, sample storage and preparation, extraction and analysis methods can influence the determination of their content in food. For these reasons literature data on the amounts of furan and furan derivatives in food often cover a wide range of values and are not easily comparable.

4. Conclusion

A HS-SPME-GC–MS method for the simultaneous determination of furan and several alkylated and oxygenated furan derivatives (2-methylfuran, 2-ethylfuran, 2-butylfuran, 2-pentylfuran, 2-acetylfuran, furfural and furfuryl alcohol) in baby food has been developed and validated. The method allowed separating the eight analytes on a polar column in about 30 min; it resulted specific for the analytes, highly accurate and reproducible with limits of detection ranging between 0.018 and 0.035 ng/g and limits of quantification between 0.060 and 0.117 ng/g, depending on the analyte. The developed method was successfully applied to investigate the content of furan and furan derivatives in several fruit- and meat-based jarred baby food samples, revealing significant quantitative differences among the samples mainly related to the fat content.

This simple, rapid and solvent free procedure could be routinely used for the analysis of furan and furan derivatives in processed baby food providing competent quantitative data functional to risk assessment.

Disclosure of potential conflicts of interest

Authors disclose that there are no financial/personal interests or believes that could have affected their objectivity.

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