RESEARCH ARTICLE

Impact of various food ingredients on the retention of furan in foods

Fien Van Lancker[1], An Adams[1], Agnieszka Owczarek[1,2], Bruno De Meulenaer[2] and Norbert De Kimpe[1]

[1] Department of Organic Chemistry, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
[2] nutriFOODchem unit, Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Since furan is classified as ''possibly carcinogenic to humans,'' many studies investigated furan concentrations in foods. However, no data are available on the impact of food ingredients on the retention or release of furan from food. These data are important, since they explain the differences in furan removal during domestic food preparation. Furan retention was studied by spiking various samples with D_4-furan and comparing D_4-furan evaporation from these samples with comparable aqueous solutions. In addition, furan concentrations were determined. Furan retention caused by starch gels was negligible. Oils caused high furan retention: peak areas of furan in oils ranged from 22 to 25% of the corresponding aqueous solutions. In addition, in coffee, furan retention was mainly caused by the lipophilic fraction. However, since furan retention was also found in defatted coffee and coffee grounds, other coffee constituents also have the ability to retain furan. Peak areas of furan in the headspace of baby foods ranged from 71 to 97% of those in water. In addition, in this case, the highest retention was found in baby foods with added oils. Baby food containing spinach showed the highest furan concentration (172 ppb) as well as the highest furan retention.

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1 Introduction

Although the presence of furan in food has been known for a long time [1], it received little attention until the report of its possible carcinogenic properties in 1995 [2]. At present, furan is classified as ''possibly carcinogenic to humans'' (Group 2B) by the International Agency for Research on Cancer [2]. Both the US Food and Drug Administration (http://www.cfsan.fda.gov/~dms/furandat.html, accessed October 23, 2008) [3] and the European Food Safety Agency [4] expressed their concerns about the presence of furan in heat-processed foods and stimulate research on this topic.

Relatively high amounts of furan have already been detected in a number of heated foods, particularly in foods which are heat-processed in cans and jars, and in strongly heated foods, such as roasted coffee [3]. In the category of canned and jarred foods, furan in baby foods causes the highest concern. Since the contribution of these foods to a baby's diet might be very extensive, the exposure to furan cannot be neglected. Furan concentrations up to 100 ppb have been detected in baby foods [3]. Exposure estimates for adults indicate brewed coffee as the major source of furan in the adult diet [5, 6]. Zoller *et al.* [6] detected concentrations up to 200 ppb in roasted coffee brew.

Several precursors have been suggested to induce furan formation upon thermal treatment, such as ascorbic acid, Maillard reaction systems, unsaturated fatty acids, as well as carotenes and organic acids [7–13]. These precursors lead to the formation of furan either directly *via* degradation of the

Correspondence: Professor Norbert De Kimpe, Department of Organic Chemistry, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium
E-mail: Norbert.DeKimpe@UGent.be
Fax: +32-9-264-62-43

Abbreviations: HMW, high molecular weight; **LMW,** low molecular weight; **SPME,** solid-phase microextraction

precursor or indirectly *via* the recombination of smaller fragments.

Besides to the mechanism of formation of furan from different precursors, current research is mainly devoted to analytical techniques and encountered problems [8, 14–18], to the occurrence of furan in foods and to the formation of furan in foods under different conditions [5, 6, 19]. In most cases, the samples are spiked with D_4-furan as internal standard. The method of adding an internal standard is preferred over using an external calibration curve, since it is well known that the matrix has a significant influence on the retention of furan.

Two different approaches have been used to determine the amount of furan in food. The first approach is to measure the amount of furan present in the food as purchased [5, 6, 19]. In the second approach, the amount of furan is measured after preparation of the food by different domestic cooking practices [15]. Also, some studies combined both approaches and compared data on the amount of furan in food before and after domestic heat treatment [20, 21]. Both approaches are useful and interesting: the first approach reveals information on the amount of furan present in different food products and thus on its precursors, while data obtained by the second approach better reflect the actual intake of furan. Since furan is a highly volatile compound (boiling point 31°C; 760 mm Hg), the decrease in furan concentrations by evaporation during domestic food preparation can be substantial. However, it is not possible to generalize the quantitative decrease in furan concentration caused by a specific procedure, since the food matrix has a significant influence on the retention of furan. Although the existence of these matrix effects is known, so far no attempt has been made to quantify these differences in furan retention. Therefore, the objectives of this study were to systematically investigate the retention or release of furan caused by several food constituents and in real foods, in order to better understand differences in furan losses during domestic food preparation and handling. The retention of furan is an important factor since it is directly correlated with the amount of furan that remains in the food and consequently with the actual intake. In addition, furan concentrations in the examined food products were determined.

2 Materials and methods

2.1 Reagents and samples

Furan (≥99%), starch from corn and starch from rice were purchased from Sigma-Aldrich (Bornem, Belgium). Starch from potatoes was purchased from Fluka. D_4-furan (99%) was purchased from Acros Organics (Geel, Belgium). Corn oil was kindly offered by Vandamme Edible Oils N.V. (Deinze, Belgium). Palm oil (crude, origin Malaysia) was kindly offered by Socfinco S.A. (Brussels, Belgium). Two kinds of olive oil (Franti, Extra Virgin, Sicilia and Carapelli Firenze, Extra Virgin, cold extraction), coffee (Arabica tradition, grinded, Carrefour), baby food samples (Olvarit), and frozen spinach (Delhaize) were obtained from the local store. Baby food ''spinach'' contained 40% potatoes, 26% spinach, 23% water, 11% apple juice, 10% rice and 2% corn oil. Baby food ''beef and vegetables'' contained 40% carrots, 18% potatoes, 18% tomatoes, 13% white beans, 10% beef and 1% corn oil. Baby food ''carrots'' contained 54% carrots, 23% potatoes, 16% water and 7% rice. Baby food ''garden vegetables'' contained 25% rice, 20% carrots, 20% cauliflower, 13% potatoes, 13% apple juice and 9% water. Methanol (>99.99%) was of analytical grade.

2.2 Materials

Dialysis tubings (Sigma-Aldrich), supplied in rolls, dry; cellulose membrane, cutoff 12 000 Da; average flat width, 34 mm; average diameter, 21 mm; capacity, 110 mL/ft.

2.3 Preparation of the samples

2.3.1 Starches

One gram of starch was mixed with 20 mL of water. To ensure gelatinization, the flasks were placed in a water bath at 90°C for 10 min under constant stirring. The concentration of starch used to prepare the starch gels was chosen to ensure that the gels were not completely solid after gelatinization. The resulting gels were cooled before spiking.

2.3.2 Oils

No special treatment was needed to determine the furan concentrations in the oils. Before studying the influence of oils on the retention of furan, all oils were flushed with N_2 for 30 min to remove most of the volatiles. In this way, lower peak areas caused by competition with other volatiles for fiber absorption were avoided.

2.3.3 Coffee

Coffee was prepared as follows: 50 g of grounded coffee was extracted by adding 150 mL of hot water (75°C) and stirring for 5 min [22]. The resulting mixture was filtered and the residue on the filter was extracted again with 150 mL of hot water (75°C). Both filtrates were combined and referred to as coffee brew. For the determination of furan concentrations, coffee brew was cooled immediately in ice and kept in closed, completely filled vials, before spiking with D_4-furan. To study the influence of coffee and coffee constituents on furan retention, coffee brew was defatted by extraction with

dichloromethane (two times 100 mL). Afterwards, the defatted coffee was fractionated in two fractions: a low molecular weight (LMW) fraction (<12 000) and a high molecular weight (HMW) fraction (>12 000) by dialysis. A dialysis tube was filled with 100 mL of coffee brew which was dialyzed against water at 4°C until the surrounding liquid was colorless. Subsequently, both LMW and HMW fractions were lyophilized and redissolved in water at 5 mg/mL.

Experiments concerning furan retention were performed with coffee brew, defatted coffee brew, a solution of the LMW fraction (5 mg/mL), a solution of the HMW fraction (5 mg/mL) and the coffee grounds that remained on the filter. All liquids were flushed with N_2 before spiking with D_4-furan to remove most of the volatiles. Coffee grounds were freeze-dried before spiking with D_4-furan.

2.3.4 Baby foods

For the determination of the furan concentrations, baby foods were vortexed while the jars were still closed. The jars were cooled in ice before opening. In case of furan retention studies, baby foods were incubated at 35°C for 15 min prior to stirring for 15 min at 35°C by means of an Ultra-Turrax. In this way, most of the volatiles were removed.

2.3.5 Spinach

To determine the formation of furan from spinach, frozen spinach was defrosted at room temperature. Afterwards, the spinach leaves were patted dry and 1 g of spinach was weighed into a 20 mL headspace vial. The vial was closed and heated for 20 min at 121°C (1.2 bar) to simulate sterilization conditions of baby food. After sterilization, the vials were immediately cooled in ice. To determine the amount of furan formed, the samples were spiked with 50 μL D_4-furan working solution.

2.4 Preparation of the standards

Stock solutions of furan and D_4-furan were prepared by adding 10 μL (D_4-) furan *via* a gastight syringe through the septum of a weighed 20 mL headspace vial (Gerstel, Mülheim a/d Ruhr, Germany) containing 20 mL of methanol. The mixture was then weighed again to determine the exact concentration of furan. Stock solutions were stored at 4°C for no longer than 5 days. Working solutions were prepared daily by adding 4 μL (or 10 μL in case of baby foods) of stock solution to a 20 mL headspace vial containing 20 mL of water using the same procedure.

For the determination of the furan concentration, calibration standards were prepared daily by injecting aqueous working solutions (10, 50, 100 or 250 μL) of furan and a fixed volume (50 μL) of D_4-furan into a 20 mL headspace vial containing 700–940 μL of water by means of a gastight syringe. This yielded exactly known furan solutions ranging from 1 to 25 ng/mL in the 20 mL headspace vial (for baby food analyses 2.5 to 62.5 ng/mL). Calibration standards were kept in ice during preparation and were closed immediately afterwards.

2.5 Spiking of the samples

All samples were kept in ice during spiking and were closed as fast as possible after spiking. For the determination of furan concentrations, about 1 mL of sample in case of oils and coffee or ±0.4 g of sample in case of baby foods, was transferred to a 20 mL weighed headspace vial and spiked with 50 μL of D_4-furan working solution by means of a gastight syringe. After closing the vial, the vial was weighed again to determine the exact amount of sample, which was taken into account for future calculations. Furan was quantified by comparing the peak area ratios of the response at *m/z* 68 and 72 for the sample with that of the calibration curve. To study the furan retention caused by several matrices, exactly 1 mL of sample in case of oils, starches and coffee was transferred to a 20 mL headspace vial, or 0.2 and 1 g in case of coffee grounds and baby foods, respectively. Then, samples were spiked with 50 μL of D_4-furan working solution by means of a gastight syringe. The resulting peak areas were compared with those obtained by spiking 1 mL of water with the same quantity of D_4-furan. In case of coffee grounds, peak areas were compared with those obtained by spiking 0.2 g of sand with the same amount of D_4-furan. All samples were vortexed for at least 20 s prior to analysis to ensure homogenization and were analyzed in triplicate.

2.6 Analysis of the samples

Furan was measured by solid-phase microextraction (SPME) coupled with GC-MS. SPME experiments were carried out using a 75 μm carboxen-polydimethylsiloxane fiber (Supelco, Bornem, Belgium). For furan retention studies, samples were incubated for 30 min at 35°C prior to sampling. Afterwards, the fiber was exposed to the headspace of the samples for 1 min at 35°C. For determination of the furan concentrations, the fiber was exposed to the headspace for 25 min at 35°C. Desorption was carried out at 300°C for 5 min. SPME extraction and desorption were performed automatically by means of an MPS-2 autosampler (Gerstel).

GC-MS analyses of the SPME extracts were performed with an Agilent 6890 GC Plus coupled to a quadrupole mass spectrometer 5973 MSD (Agilent Technologies, Diegem, Belgium), and equipped with a Varian CP-PoraBOND Q capillary column (25 m length × 0.32 mm id; coating 5 μm film thickness). Working conditions were as follows: injector

300°C, transfer line to MSD 250°C, carrier gas (He) 1.2 mL/min; SPME desorption in a CIS-4 PTV injector (Gerstel) in splitless mode; ionization: EI 70 eV. The oven temperature was programmed from 50 to 260°C at 8°C/min, hold 7 min. Analyses were performed in SIM mode. Quantification was based on MS signals at *m/z* 68 for furan and *m/z* 72 for D_4-furan. The following qualifiers were used: *m/z* 39 for furan and *m/z* 42 for D_4-furan.

2.7 Statistical analysis

Differences in furan retention were statistically evaluated by means of standard deviations (n = 3) and Student's *t*-test ($\alpha = 0.05$).

3 Results and discussion

3.1 Furan retention

Since furan is a highly volatile compound, an important part of the furan formed during thermal treatment will be lost during food handling and food preparation by evaporation, depending on the food matrix. Therefore, the retention or release of furan by different food constituents was systematically evaluated. This furan retention/release was studied by spiking the samples with D_4-furan and comparing the resulting headspace peak areas by means of SPME-GC-MS analysis with those obtained from comparable aqueous solutions. D_4-furan is used to imitate the behavior of furan, since the studied samples may already contain furan, which will influence the resulting peak area. Zoller *et al.* [6] demonstrated the similar partitioning behavior of furan and D_4-furan in different food matrices. Column and fiber selection for SPME analysis of furan were based on Goldmann *et al.* [14]. To avoid distortion of the equilibrium partitioning of furan between the matrix and the headspace, a very short sampling time of 1 min was applied, since this was shown to represent equilibrium headspace conditions in comparable flavor release studies [23, 24].

The release of furan from starch gels, vegetable oils, coffee brew, coffee constituents and baby foods is depicted in Fig. 1.

It can be seen that starch had little effect on the release of furan. Moreover, the release from corn starch and rice starch gels was not significantly different from the release from water. It is known, however, that specific carbohydrates, such as starch, consist of three-dimensional structures with hydrophobic regions and are capable of forming inclusion complexes with various hydrophobic volatiles [25]. Only in case of starch from potatoes, a small retention of furan was detected (94% of the release from water). In this context, it must be mentioned that the viscosity of the gel from potato starch was clearly higher than the viscosity of both other gels. Therefore, in this case the significantly higher retention can probably be explained by a texture effect. Texturing agents have been reported to influence release of flavor volatiles by increasing the viscosity, often resulting in a significant decrease in perceived flavor [26]. However, since the viscosity of potato starch gel was relatively high (almost solid) and only a low retention was detected, it can be concluded that the viscosity of the matrix does not influence the retention of furan to a high extent.

In contrast to the almost negligible retention of furan by starches, oils clearly caused major decreases in furan release (Fig. 1). Lipids are the food ingredients that usually have the biggest effect on the partitioning of flavor compounds between products and the gaseous phase [27]. For example, higher amounts of fats and oils generally lower the volatility of hydrophobic odorants such as long-chain aldehydes [28]. The peak areas of D_4-furan in oils as compared with water ranged from 22 to 25%. These results imply that the presence of oils in foods decreases the volatilization of furan and may as such increase the actual intake of furan to a large extent. Becalski *et al.* [19] also reported a decrease of 30% in the response of furan standards when adding 4% of corn oil to water. This observation can be explained by the

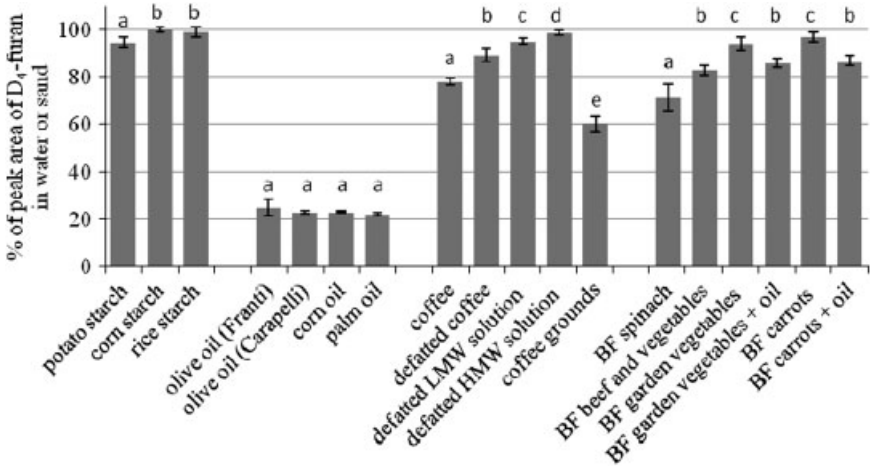

Figure 1. Release of D_4-furan from different matrices (BF = baby food). Values represent the comparison (%) of headspace peak area above a food product with the corresponding peak area above water (or sand in case of coffee grounds). Error bars represent standard deviations ($n = 3$). Significant differences ($p < 0.05$) are represented by different letters (a–e) between samples within one food matrix group. In general, especially lipophilic compounds caused a significant retention in D_4-furan.

lipophilic characteristics of furan. No significant differences were found between the examined oils, although the composition (and degree of saturation) of the oils was very different: corn oil is highly unsaturated, palm oil is highly saturated (solid at 20°C) and both olive oils have an intermediate saturation. Therefore, it is concluded that all oils have a similar influence on the retention of furan, regardless their degree of (un)saturation.

Furan retention studies were also conducted with coffee, since it is believed to be the major source of furan in an adults diet. As can be seen in Fig. 1, coffee brew also significantly retained D_4-furan in solution. The peak areas were 78% of those obtained from water. To evaluate which constituents of coffee influenced the retention of furan the most, coffee brew was defatted and afterwards fractionated in a low ($<12\,000$) and high ($>12\,000$) molecular weight (LMW and HMW, respectively) fraction. Defatted coffee brew showed a significantly lower retention of D_4-furan than coffee brew: the D_4-furan response increased significantly from 78 to 89% after defatting. This observation is in agreement with the conclusions stated above concerning the interaction between lipophilic compounds and furan. D_4-furan peak areas obtained from the LMW and HMW coffee solution (5 mg/mL) were 95 and 99% of corresponding aqueous solutions, respectively. Furan release from HMW coffee solutions was not significantly different from the release from water. This implies that furan is retained slightly more by the LMW fraction, which is the major fraction of coffee. In addition, release of D_4-furan by coffee grounds was compared with the release of D_4-furan by sand. The release of D_4-furan from coffee grounds was only 60% of the release from sand.

The presence of furan in baby foods has become a subject of great concern, since the contribution of these foods to a baby's diet might be very extensive. Therefore, four baby foods (''spinach,'' ''carrots,'' ''beef and vegetables'' and ''garden vegetables'') were also included in this study to get a better view on the differences in furan retention. Peak areas of D_4-furan in baby food were also compared with peak areas in aqueous solutions. Although it might seem better to use a more viscous matrix as a reference, water was selected as the reference, since the experiments with starches revealed only a minor influence of the viscosity of the matrix on furan release. As can be seen in Fig. 1, baby food ''carrots'' and baby food ''garden vegetables'' did not retain furan to a high extent, although there was a significant retention as compared with water. The peak areas of D_4-furan in these foods were 97 and 94% of those in water, respectively. The retention of D_4-furan was significantly higher in baby food ''beef and vegetables'' and even more in baby food ''spinach'', which revealed responses of 83 and 71%, respectively. Strikingly, baby food ''spinach'' and baby food ''beef and vegetables'' contained 2 and 1% corn oil, respectively, whereas baby food ''carrots'' and ''garden vegetables'' contained no added oils. Although the total fat content of baby food ''beef and vegetables'' (3.4%) was higher than the total fat content of baby food ''spinach'' (1.9%), the retention of D_4-furan by baby food ''spinach'' was significantly higher than the retention by baby food ''beef and vegetables.'' In addition, the total amount of unsaturated fat was higher in baby food ''beef and vegetables''. These results lead to the assumption that the addition of a low amount of oil significantly increased the retention of furan, and this to a much higher extent than the total fat content. Therefore, 2% of corn oil was added to baby food ''carrots'' and baby food ''garden vegetables'' to study whether this would cause a decrease in D_4-furan release. These results are also depicted in Fig. 1. As expected, the peak area ratios of baby food ''carrots'' and baby food ''garden vegetables'' significantly decreased from 97 to 87% and from 94 to 86%, respectively, by adding 2% of corn oil. However, the obtained responses were not as low as those obtained from baby food ''spinach''. This is probably due to other constituents with lipophilic characteristics that influence the release of furan.

3.2 Furan concentrations

Besides the furan retention, also the furan concentrations of all the investigated food products (without any additional thermal treatment) were studied. The results are depicted in Table 1. No furan was detected in the investigated starches.

For oils, furan concentrations were similar in the two types of olive oil and in corn oil (2.3–9.0 ng/g). Palm oil contained significantly more furan (64.4 ng/g). It is believed that the high amounts of carotenoids in crude palm oil (500–700 ppm [29]) are responsible for this difference, since carotenoids were identified as important precursors of furan by Becalski and Seaman [8]. Regarding these furan concentrations in oils, it should be mentioned that the oils were not fresh. Therefore, it is possible that furan had been formed by lipid oxidation and has accumulated over time. However, the oils were not heat-processed before analysis. When oil-containing food products are thermally processed, for instance, during sterilization or pasteurization, furan

Table 1. Furan concentrations in food constituents and foods

Matrix	Furan (ng/g)	
Olive oil (Franti)	9.0±0.2	a
Olive oil (Carapelli)	4.0±0.6	b
Corn oil	2.3±0.3	b
Palm oil	64.4±1.5	c
Freshly brewed coffee	19.2±0.3	d
Baby food ''spinach''	172.7±8.0	e
Baby food ''beef and vegetables''	83.3±8.2	c
Baby food ''garden vegetables''	75.1±1.7	c
Baby food ''carrots''	100.2±5.1	f

Values given are averages of triplicate measurements ± the standard deviation. Significant differences ($p<0.05$) are represented by different letters (a to f).

concentrations due to the presence of oils will probably be higher than those reported here, since furan is easily formed from fatty acids at elevated temperatures [8]. Becalski and Seaman [8] reported furan concentrations of 125 to 625 ng/g for linoleic and linolenic acid, respectively, after heating for 30 min at 118°C.

Combination of the results on furan retention and furan concentrations clearly demonstrates the contribution of oils in foods to furan exposure. On the one hand, oils are precursors of furan, and on the other hand, the presence of oils in food largely increases the retention of furan. This implies that the presence of oils influences the actual intake of furan *via* two different processes. As, from a nutritional point of view, elimination of oils from baby food is not an option, it would be better to add the oils after heat-processing, right before consumption of the baby food.

Freshly brewed coffee contained 19.2 ng furan *per* gram of sample. The Food and Drug Administration [3] reported concentrations of furan ranging from 33.6 to 84.2 ng/g in different types of brewed coffee. Furan concentrations of 13 to 199 ng/g were detected in coffee brew by Zoller *et al.* [6]. These authors detected the lowest concentrations of furan when coffee was prepared by means of filtration and the highest furan concentrations when coffee brews were prepared with an espresso-type machine. This is in agreement with the rather low amounts of furan found in coffee in this study since the coffee was prepared by means of filtration.

Concentrations of furan detected in baby foods are depicted in Table 1 as well. The highest concentration of furan found in baby food (172.7 ng/g) was found in baby food ''spinach.'' Since all ingredients except spinach are also present in at least one of the other baby foods, this high furan concentration is most likely caused by the spinach itself. To test this hypothesis, 1 g of spinach was heated for 20 min at 121°C (1.2 bar) in a 20 mL vial to simulate sterilization conditions. Before sterilization, no furan was detected, while after sterilization, the amount of furan present in the sample was 113.9 ± 13.4 ng/g. These results clearly indicate that furan can be formed from spinach in relatively high amounts. So far, no data on furan concentrations in spinach-based foods have been reported, to the best of our knowledge. Vitamin C or carotenoids can be possible precursors of furan in spinach. Besides the high furan concentration, spinach-containing baby food also revealed the highest retention of furan.

High furan concentrations were also detected in baby food ''carrots'' (100.2 ng/g). The presence of carotenoids in carrots is often referred to as an important source of furan. Concentrations of furan reported in baby food containing carrots typically ranged from 30 to 60 ng/g [3, 6]. In this study, higher amounts were detected. Baby food ''beef and vegetables'' and baby food ''garden vegetables'' revealed slightly lower furan concentrations: 83.3 and 75.1 ng/g, respectively. However, these furan concentrations are also higher than most concentrations reported before [3, 6].

4 Concluding remarks

Due to the high volatility of furan, volatilization during food handling and food preparation is important in the removal of furan from food. This furan volatilization, however, is strongly influenced by the food matrix. In this study, the differences in furan retention caused by several food constituents were systematically studied. It was shown that the release of furan is very different depending on the food matrix. As can be expected, especially lipophilic compounds, such as oils, caused a significant retention of furan. However, since not all results can be explained by interaction of furan with the lipophilic fraction (for example, in defatted coffee), other food constituents also have the ability to retain furan. This study clearly illustrates that besides the original furan concentrations, also its retention by the food matrix should be taken into account in risk evaluation since it will determine the amount of furan removed from the food product by volatilization.

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