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Occurrence of furan in commercial processed foods in Brazil

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Occurrence of furan in commercial processed foods in Brazil

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Selected commercial processed foods available in the Brazilian market (306 samples) were analysed for furan content using a validated gas chromatography-mass spectrometry method preceded by headspace solid phase micro-extraction (HS-SPME-GC/MS). Canned and jarred foods, including vegetable, meat, fruit and sweet products, showed levels up to $32.8 \,\mu g \, kg^{-1}$, with the highest concentrations observed in vegetables and meats. For coffee, furan content ranged from 253.0 to $5021.4 \,\mu g \, kg^{-1}$ in the roasted ground coffee and from not detected to $156.6 \,\mu g \, kg^{-1}$ in the beverage. For sauces, levels up to $138.1 \,\mu g \, kg^{-1}$ were found. In cereal-based products, the highest concentrations (up to $191.3 \,\mu g \, kg^{-1}$) were observed in breakfast cereal (corn flakes), cracker (cream crackers) and biscuit (wafer). In general, these results are comparable with those reported in other countries and will be useful for a preliminary estimate of the furan dietary intake in Brazil.

Keywords: GC/MS; process contaminants; canned foods; coffee; bakery products

Introduction

Furan is a highly volatile and lipophilic organic compound that can be formed in foods as a result of heating or exposure to ionising or ultraviolet radiation (Codex Committee on Contaminants in Foods (CCCF) 2011). The occurrence of furan and its derivatives in heat-treated foods such as coffee, canned meat, bread and cooked chicken has long been recorded (Maga 1979).

Furan represents a potential concern since it was classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC) (1995), based on the increased incidence of liver tumours and cholangiocarcinomas in rats and mice at high exposure doses (National Toxicology Program (NTP) 1993). It is assumed that *cis*-2-butene-1,4-dial, the reactive metabolite of furan, is the causative agent leading to toxicity and carcinogenicity in experimental animals. However, epidemiological studies investigating a possible association between furan exposure and human cancer have not been reported (Bakhiya and Appel 2010).

In 2004, researchers from the US Food and Drug Administration (USFDA) reported the occurrence of furan in a broad variety of thermally processed foods, indicating significant concentrations in coffee, in foods that underwent a heat treatment in sealed containers including baby foods, as well as in crusty and dry products such as toasted bread or bread crust (USFDA 2004). These findings were later confirmed by the European Food Safety Authority (EFSA) in a report containing a compilation of available data (EFSA 2004). According to these preliminary results, coffee contained the highest concentrations of furan, up to $5050 \,\mu g \, kg^{-1}$ for roasted beans, while levels up to $112 \,\mu g \, kg^{-1}$ were reported for jarred baby foods.

The presence of furan in an unexpected number of commonly consumed foods caused a worldwide concern regarding the potential risks of this contaminant to human health since preliminary exposure assessments revealed a relative small margin between the estimated intakes and furan doses which induce liver tumours in experimental animals (EFSA 2004). As a consequence, several research studies have been led by scientists and industry on analysis, formation and control, while advice on consumption has been communicated (CCCF 2011).

To date it has been well established that gas chromatography/mass spectrometry (GC/MS) preceded by headspace sampling (HS) or headspace solid phase micro-extraction (HS-SPME) are the most appropriate techniques to determine furan in foods (Becalski et al. 2005; Goldman et al. 2005; Bianchi et al. 2006; Hasnip et al. 2006; USFDA 2006; Altaki et al. 2007; Wenzl 2008). Moreover, several compounds such as carbohydrates, amino acids, polyunsaturated

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fatty acids and ascorbic acid have already been identified as potential precursors in the generation of this contaminant (Locas and Yaylayan 2004; Becalski and Seaman 2005; Märk et al. 2006; Limacher et al. 2007). On the other hand, more information is still needed regarding the mechanism of furan formation in foods as are practical and consistently effective strategies for decreasing its occurrence levels (CCCF 2011).

In addition, action plans and monitoring programmes were proposed by several countries (USFDA 2005; European Commission 2007). In the United States, the USFDA planned actions mainly on analytical methods, exposure assessment and risk analysis (USFDA 2005). In Europe, data from the European Union member states collected between 2004 and 2010 were organised by EFSA in a public database (EFSA 2011). Independent surveys have also been conducted in several European countries (Zoller et al. 2007; Bononi and Tateo 2009; Jestoi et al. 2009; Wegener and López-Sánchez 2010; Scholl et al. 2012) as well as in Canada (Becalski et al. 2010), Australia (Food Standards Australia New Zealand (FSANZ) 2008), and Asian countries (Kim et al. 2010; Liu and Tsai 2010).

In Brazil, a survey carried out to determine furan levels in commercial baby foods showed results comparable with those obtained in other countries (Arisseto et al. 2010). However, no data for commercially processed foods from Brazil other than baby foods have been published so far. Therefore, the objective of this study was to use a validated HS-SPME-GC/MS method to collect information on furan levels in canned/jarred foods, sauces, coffees and cereal-based products in order to provide a comprehensive database on the occurrence of this contaminant in Brazilian foods.

Materials and methods

Standards and chemicals

Furan and $[{}^{2}H_{4}]$ furan (furan-d₄) were obtained from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA) at purity higher than 98%. Methanol was of HPLC grade (Tedia Company Inc., Fairfield, OH, USA) and water was purified by reverse osmosis (Gehaka, São Paulo, SP, Brazil). Individual stock solutions of both standards at approximately 2 mg ml^{-1} were prepared by dissolving the compounds in methanol. Intermediate (approximately 20 µg ml^{-1}) and work solutions (approximately $2 \text{ and } 0.2 \text{ µg ml}^{-1}$) were prepared in water.

Samples

A total of 306 samples of canned/jarred foods, sauces, coffees and cereal-based products were purchased at

supermarkets, restaurants and cafeterias in the city of Campinas, SP, Brazil, between September 2009 and September 2011. The sampling was made considering previous data reported by other countries and included products typically consumed in Brazil, commercially available in metal cans, such as feijoada (a thick stew made from black beans and a variety of salted pork and beef products), doce de leite (a thick, caramel-like sweet made from sugar and milk) and goiabada (a conserve made of guava, sugar and water). For most of the products, three different brands and two different lots of each brand were analysed. The samples were stored at 4°C for at least 4h before homogenisation, which was carried out as quickly as possible in order to avoid furan losses due to volatilisation.

Determination of furan

Furan was determined by using a HS-SPME-GC/MS method as described previously (Arisseto et al. 2010, 2011). The sample weight, volume and concentration of furan-d₄ working solution, volume of water and extraction temperature were optimised for each group of sample, and are shown in Table 1. The SPME was carried out for 30 min employing a 75 µm carboxen-polydimethylsiloxane (CAR-PDMS) fibre (Supelco, Bellefonte, PA, USA). The GC/MS system consisted of a HP 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a MSD 5973 mass spectrometer (Agilent Technologies) and the chromatographic conditions were as previously described. The mass spectrometer was operated in positive electron impact ionisation mode (+EI) with 70 eV of electron energy. Selected ion monitoring (SIM) was applied by using three characteristic ions for furan (m/z 68, 39 and 69) and two characteristic ions for furan-d₄ (m/z 72 and 42). The quantification of furan in samples was proceeded by extrapolation from a linear analytical curve using furan-d₄ as an internal standard.

Method validation

The method was validated in terms of linearity, selectivity, limit of detection (LOD), limit of quantitation (LOQ), trueness (recovery) and precision (repeatability and within-laboratory reproducibility) according to the guidelines laid down by the Instituto Nacional de Metrologia, Normalização e Qualidade Industrial (INMETRO) (2007). Linearity was evaluated by using at least six calibration points. Selectivity was determined by comparison between curves set on standard solutions and on matrix by applying the F-test (Snedecor) and t-test (Student). LOD and LOQ were estimated by seven independent replicates of the matrix and calculated as three- and 10-fold the

Sample	Weight (g)	IS volume (µl)	IS concentration $(\mu g m l^{-1})$	Water (ml)	Extraction temperature (°C)
Canned/jarred foods	1.0	125	0.2	1	25
Coffee (beverage) and sauces	1.0	125	0.2	_	25
Cereal-based products	1.0	125	0.2	5	25
Coffee (powder)	0.25	150	2.0	1	35

Table 1. Parameters (sample weight, volume of internal standard (IS), concentration of IS, volume of water and extraction temperature) used in the solid-phase micro-extraction (SPME) for each group of sample.

Table 2. Validation results.

Matrix	Calibration range (µg kg ⁻¹)	r^2	Matrix effect	$\begin{array}{c} \text{LOD} \\ (\mu g k g^{-1}) \end{array}$	$\begin{array}{c} LOQ \\ (\mu g k g^{-1}) \end{array}$	R (%)	CV _r (%)	CV _R (%)
Baby food	0-100	0.998	No	0.7	2.4	80–107	5.6-9.4	7.4–12.4
Coffee	0-9600	0.992	No	3.0^{a}	$10.0^{\rm a}$	76-101	1.7 - 7.1	6.2-13.8
Soy sauce	0-100	0.998	No	1.3	4.2	83-127	2.8-13.2	17.0-23.6
Bread	0-100	0.998	No	1.4	4.8	92-123	6.7–9.8	10.3-23.3

Notes: ^aLimits estimated for beverages.

 r^2 , coefficient of correlation; LOD, limit of detection; LOQ, limit of quantification; *R*, recovery; CV_r, coefficient of variation under repeatability conditions (same day); and CV_R, coefficient of variation under within-laboratory reproducibility conditions (different days).

standard deviation of the obtained responses, respectively. Recovery, repeatability and within-laboratory reproducibility were evaluated by spiking the matrix with furan at three different levels (seven replicates for each level). The validation procedure was performed for different matrices such as jarred baby food (containing vegetables, beef, rice and pasta), ground roasted coffee, soy sauce and whole bread in order to represent most of the analysed foods. If a blank matrix was not available, a sample with a low contamination level was used in the experiments.

Results and discussion

Method validation

Previous studies (Arisseto et al. 2010, 2011) have described in detail the definition of chromatographic conditions and the optimisation of the SPME procedure for baby food and coffee matrices, which were taken into account in the present work.

The results obtained during the validation experiments are summarised in Table 2. As can be seen, the method is reliable and can be applied to determine furan in several foodstuffs. The method also showed good linearity in the calibration ranges tested as well as high selectivity, since no matrix effects were observed for the evaluated samples. The estimated LOD and LOQ were appropriate to detect low concentrations of furan. Mean recoveries varied from 76% to 123%, and the coefficients of variation ranged from 1.7% to 13.2% for repeatability and from 6.2% to 23.6% for within-laboratory reproducibility, which are within the tolerances considered acceptable (Horwitz et al. 1980; European Commission 2002).

Occurrence in food

The validated HS-SPME-GC/MS method was used to determine the concentrations of furan in canned/jarred foods, coffees, sauces and cereal-based products available on the Brazilian market. Although the sampling included brands that are probably commercialised in other Brazilian regions, it should be noted that the samples were only collected in the Campinas area and, therefore, may not be representative of the national food supply. However, this study provides a comprehensive database on furan levels in a selected segment of Brazilian food resources.

Canned/jarred foods

The results obtained in relation to the occurrence of furan in canned and jarred foods are presented in Table 3. The concentrations ranged from not detected to $32.8 \,\mu g \, kg^{-1}$. The mean values were calculated at $4.9 \,\mu g \, kg^{-1}$ for vegetable products, $10.9 \,\mu g \, kg^{-1}$ for

			Furan ($\mu g k g^{-1}$)	
Group	Sample	N/N+	Mean	Minimum– maximum
Vegetables	Mixed vegetables Tomato puree Tomato sauce Olive Mushroom Heart of palm Maize Pea	5/1 6/4 6/3 6/5 6/4 6/4 6/5 4/3	$\begin{array}{c} 0.6 - 1.5 \\ 5.3 \\ 6.0 \\ 5.0 \\ 2.8 \\ 8.5 \\ 6.8 \\ 3.4 \end{array}$	$\begin{array}{r} \text{n.d.}-3.1 \\ < 2.4-13.1 \\ < 2.4-15.7 \\ < 2.4-7.5 \\ < 2.4-7.5 \\ < 2.4-16.1 \\ < 2.4-13.0 \\ < 2.4-5.5 \end{array}$
Meats	Feijoada Tuna Sausage Sardine Ham pate	5/4 6/5 4/4 6/6 3/3	6.7 5.3 10.8 9.4 22.5	< 2.4-16.5 < 2.4-9.2 4.8-18.8 2.7-15.5 17.1-32.8
Fruits and sweets	Peach Pineapple Strawberry jam Doce de leite Goiabada	6/1 5/4 6/2 4/3 4/3	0.9–1.8 6.8 1.9–3.5 3.7 2.7	n.d5.3 <2.4-10.3 <2.4-7.8 <2.4-5.9 <2.4-3.3

Notes: *N*, number of samples; *N*+, number of samples above the limit of quantification (LOQ = $2.4 \,\mu g \, \text{kg}^{-1}$); and n.d., values below the limit of detection (LOD = $0.7 \,\mu g \, \text{kg}^{-1}$). To calculate the mean, values below the LOD and LOQ were

treated according to World Health Organization (WHO) (2002). The range indicates different results for the lower and upper bounds for a group of data with fewer than 40% of quantifiable results.

meat products and $3.4 \,\mu g \, k g^{-1}$ for fruits and sweets. The group of meat products presented the higher percentage of positive samples (92%). The results are comparable with those reported in other studies (Zoller et al. 2007; Morehouse et al. 2008; EFSA 2011).

In general, higher concentrations of furan have been found in canned/jarred vegetables and meats. Some authors attributed this trend to the higher pH of vegetables, which could lead to a higher degradation of ascorbic acid and subsequent furan formation (Mesías-Garcia et al. 2010). Other studies demonstrated that the degradation of ascorbic acid can also be enhanced in the presence of lipids, which could constitute an important route for furan formation in foods (Owczarek-Fendor, De Meulenaer, Scholl, Adams, van Lancker, Yogendrarajah, Uvtterhoeven, et al. 2010). In addition, other factors such as availability of oxygen and the presence of buffers (phosphate, citrate) can interfere on the concentration of the contaminant in the final product (Owczarek-Fendor, De Meulenaer, Scholl, Adams, van Lancker, Yogendrarajah, Eppe, et al. 2010).

Coffees

Furan levels in coffee samples are presented in Table 4. The concentrations ranged from 253.0 to $5021.4 \,\mu g \, kg^{-1}$ in roasted ground coffee and from not detected to $156.6 \,\mu g \, kg^{-1}$ in the beverage, which is comparable with data reported in the literature (Zoller et al. 2007; Morehouse et al. 2008; Crews et al. 2009; EFSA 2011). With the exception of instant coffee (beverage), all other coffee samples showed 100% of quantifiable results. The mean concentrations observed

Table 4. Furan levels in coffee (N = 79).

			Furan (µg kg ⁻¹)		
Group	Sample	N/N+	Mean	Minimum–maximum	
Roasted ground coffee (in pillow packs)	Instant	8/8	448.9	253.0–1005.3	
	Classic	7/7	1669.7	1139.3–2026.4	
	Extra-strong	6/6	1556.1	1262.0–1791.8	
Roasted ground coffee (vacuum packed)	Classic	6/6	3472.0	2704.1–5021.4	
	Strong	4/4	4164.3	3475.9–5017.8	
	Extra-strong	6/6	2445.0	1732.1–4481.7	
	Premium	4/4	1789.4	1273.8–2328.5	
Beverage	Instant ^a	8/2	3.8–9.6	n.d20.6	
	Filtered ^b	10/10	25.5	15.4-55.1	
	Espresso	14/14	79.8	44.5-156.6	
	Cappuccino	6/6	40.7	27.7-74.2	

Notes: ^aBeverages prepared in the laboratory by using 2g of instant coffee to 100 ml water.

^bInclude beverages collected in restaurants and prepared in the laboratory (manually and automatically) by using 10 g of roasted ground coffee to 100 ml water.

N, number of samples; *N*+, number of samples above the limit of quantification ($LOQ = 10 \,\mu g \, kg^{-1}$); and n.d., values below the limit of detection ($LOD = 3 \,\mu g \, kg^{-1}$).

To calculate the mean, values below the LOD and LOQ were treated according to World Health Organization (WHO) (2002). The range indicates different results for the lower and upper bounds for a group of data with fewer than 40% of quantifiable results.

were $1224.9 \,\mu g \, kg^{-1}$ for roasted ground coffee packed in pillow packs, $2967.7 \,\mu g \, kg^{-1}$ for roasted ground coffee vacuum packed and $38.2 \,\mu g \, kg^{-1}$ for beverages.

The high levels of furan found in roasted ground coffee may be probably due to the temperatures used in the roasting process, which are generally higher than those employed in most of other thermal treatments. Within these samples instant coffee showed the lowest concentrations of furan, which could be attributed to the volatilisation of the contaminant during the extraction and concentration steps employed to obtain this product. As expected, the highest levels were observed in vacuum-packed roasted ground coffee, since the loss of volatile compounds during the storage is less significant in this type of packaging. The intensity of coffee (classic, strong and extrastrong) could not be correlated with the level of furan, although some studies have suggested that the concentration of the contaminant increases with the roasting degree (Guenther et al. 2010; Arisseto et al. 2011). The composition of the products in relation to species (C. arabica and C. canephora) could also contribute to the observed differences, since experiments carried out with coffees of light, medium and dark roasting degrees showed higher furan levels for the C. canephora species (Arisseto et al. 2011).

In contrast to roasted ground coffee, much lower concentrations were found in coffee beverages, which might be associated to furan volatilisation due to the high temperature of water as well as its solubility in this solvent. The variation on furan content in different beverages depends on the brewing procedure (filtration, percolation, pressure, etc.), on the furan content in roasted ground coffee and on the proportion of powder and water used (Kuballa et al. 2005; Zoller et al. 2007; Arisseto et al. 2011). Espresso coffees have higher furan levels due to the largest amount of powder needed to prepare this beverage, to the closed system of commercial machines that limits losses by volatilisation, to the higher pressure during brewing which increases the extraction of furan from the coffee powder, and to the reduced loss of furan in the cup since this procedure required less time for preparation (Kuballa et al. 2005; Zoller et al. 2007; Altaki et al. 2011).

It has been reported in the literature that brewed coffee is the most important contributor to furan exposure in the adult population (Morehouse et al. 2008; EFSA 2011). The choice of a brewing method that results in lower furan levels could be, in principle, a useful approach to reduce furan exposure from coffee. However, the amount of furan ingested through drinking coffee will depend not only on the final concentration of the contaminant in the beverage, but also on the serving size (Stadler 2011). Thus, it is important to note that although furan concentrations are relatively higher in espresso coffee, the usual serving size of this beverage is generally smaller in comparison with other types of coffee.

Sauces

Table 5 presents the levels of furan found in soy sauces and related products. As can be noted, all samples showed results above the LOQ, and the concentration of furan varied from 6.0 to $138.1 \,\mu g \, kg^{-1}$. Similar results were reported in other studies (USFDA 2004; EFSA 2011).

There is no information available in the literature about the possible contamination sources of soy sauce with furan. One hypothesis is that the occurrence of the contaminant be due to the use of roasted grains (such as wheat, maize and rice) in the formulation of these products. The addition of caramel colouring in some brands may also be a potential source of furan. The information available on the labels regarding ingredients and manufacturing process was not sufficient to explain the variation observed in the results and it was not possible to establish a correlation between the brand or type of soy sauce with furan levels.

Cereal-based products

Table 6 shows the results obtained for furan in cerealbased products. Of the 71 analysed samples, 62 presented quantifiable results, with concentrations up to 191.3 μ g kg⁻¹. The highest levels of the contaminant were found in breakfast cereal (corn flakes), cracker (cream crackers) and biscuit (wafer), while the lowest concentrations were observed in loaf bread and cake. Data reported by Fromberg et al. (2009) showed levels of furan up to 387 μ g kg⁻¹ in breakfast cereals. Other studies have also confirmed the occurrence of high concentrations of the contaminant in cereal-based

Table 5. Furan levels in soy sauces and related products (N=62).

		Furan ($\mu g k g^{-1}$)		
Soy sauce	N/N+	Mean	Minimum– maximum	
Traditional	26/26	27.2	10.5-57.8	
Light	7/7	21.9	7.6-37.8	
Premium	9/9	26.3	6.0-47.3	
Other	4/4	50.2	40.9-65.1	
Sauces containing soy sauce ^a	16/16	42.7	7.6–138.1	

Notes: ^aYakissoba, shimeji, oriental, tonkatsu and tare sauces.

N, number of samples; and *N*+, number of samples above the limit of quantification $(LOQ = 4.2 \, \mu g \, kg^{-1})$.

		Furan	$(\mu g k g^{-1})$
Sample	N/N+	Mean	Minimum– maximum
Granola	6/6	18.8	11.8-23.9
Cereal bar	5/5	13.2	7.6-19.4
Corn flakes	6/6	77.1	36.5-145.0
Loaf bread	6/3	5.9	<4.8-12.0
Whole bread	6/4	12.5	<4.8-29.5
French bread	6/6	26.4	5.9-50.3
Toast	6/6	42.2	24.6-85.2
Cracker (cream cracker)	6/6	95.9	42.4-166.4
Cracker (other)	6/6	22.2	15.6-29.0
Biscuit (wafer)	6/6	105.3	29.5-191.3
Biscuit (milk)	6/6	38.1	23.8-67.2
Cake (orange)	6/2	1.8 - 5.0	n.d5.6

Notes: *N*, number of samples; *N*+, number of samples above the limit of quantification (LOQ = $4.8 \,\mu g \, \text{kg}^{-1}$); and n.d., values below the limit of detection (LOD = $1.4 \,\mu g \, \text{kg}^{-1}$). To calculate the mean, values below the LOD and LOQ were treated according to World Health Organization (WHO) (2002). The range indicates different results for the lower and upper bounds for a group of data with fewer than 40% of quantifiable results.

products such as biscuits, crackers and crispbread (Hoenicke et al. 2004; EFSA 2011).

There are few studies available on the formation of furan in cereal-based products. Fromberg et al. (2009) reported that the colour of toasted bread is directly proportional to the level of furan, with the highest concentrations of the contaminant obtained for darker toasted bread. It has also been demonstrated that higher levels of furan are found in the bread crust (Zoller et al. 2007).

According to Table 6, a large variation on furan levels was observed between different products and between different brands for the same product. These differences may be related to the ingredients used in the formulation, the type of process employed and the heterogeneity of furan formation in the food (Fromberg et al. 2009; Wegener and López-Sánchez 2010).

Conclusions

This paper reports the results of a survey on furan content in 306 samples collected from the Brazilian market, including canned/jarred foods, coffees, sauces and cereal-based products. Furan was found in quantifiable amounts in most of the samples, in levels that are comparable with those obtained in other countries. Coffee, which has been identified as the most important contributor to furan exposure, presented very high concentrations in the powder, but much lower levels in the beverages. Significant contents were also found in cereal-based products, which can constitute another important source of furan in the diet taking into account their high and usual consumption. These results will be used for a preliminary estimate of the furan dietary intake in Brazil and it is expected that potential alternatives to reduce furan exposure can be derived from these data, since the scientific literature has indicated that mitigation strategies are mostly targeted at the level of the consumer, rather than production methods.

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