TITLE: Effect of frying process on furan content in foods and assessment of furan exposure of Spanish population

AUTHORS: Isabel Juániz, Concetta Zocco, Vanessa Mouro, Concepción Cid and M. Paz De Peña*

Published in LWT-Food Science and Technology 68, 549-555 (2016)

DOI: http://dx.doi.org/10.1016/j.lwt.2015.12.061

Department of Nutrition, Food Science and Physiology, School of Pharmacy, University of Navarra. IdiSNa, Navarra Institute for Health Research. E-31080-Pamplona, Spain.

*Corresponding author: María-Paz de Peña. Tel: +34 948 425600 (806580); Fax: +34 948 425740. E-mail address: mpdepena@unav.es

ABSTRACT

Furan content in eight bread-coated frozen foods (ham croquettes, squid rings, tuna pasties, churros, nuggets, fish fingers, onion rings and san jacobos) deep-fried in fresh and reheated olive oil, and in five cooked vegetables was evaluated. Deep fried foods showed the highest levels of furan between 12 µg kg^{-1} (tuna pasties) and 172 µg kg^{-1} (onion rings), with a furan increase tendency when reheated oil was used. In vegetables, furan was only found at low level in griddled onion (3.5 µg kg^{-1}). The lower temperature applied (< 150ºC) in comparison to that of the deep-fried foods (190 ºC), the furan volatilization during longer time cooking (15 min vs 6 min) together with the food composition differences might explain the low furan content in vegetables. As a preliminary approach for risk assessment, the margin of exposure (MOE) was calculated. The total daily intake of furan by Spanish population (239-4372 ng/kg bw/day) with MOEs below 10,000 indicates a human public health concern. However, MOEs for fried foods showed that furan could suppose a possible health risk only in people with a high consumption of these products. Nevertheless, further studies should be developed to provide furan exposure data of other fried foods.

KEYWORDS: Furan; Deep-Frying; Cooking; Processing contaminants; Risk assessment

1. Introduction

Frying is a culinary process applied to a great variety of foods. Innovation in the food industry with the development of new food products associated to social changes in Western countries have increased the consumption of a great variety of time-saving "ready-to-heat" frozen foods. In Spain, the consumption of pre-cooked frozen foods, most of them bread-coated, increased by 7.8% in the period 2001-2006 (MAGRAMA, 2006). When considering both household and catering and institutions consumption, recent data (2014) indicate that around 12.3 kg per capita per year of ready-to-serve foods (including pre-cooked frozen foods) were consumed in Spain, increasing every year (0.4% higher than in 2013) (MAGRAMA, 2014). Croquettes and pasties account for around 20% of precooked foods with high amount of cereals. Churros are also a typical Spanish fried food product with a high consumption among cereal products (1.32 g/capita/day) (AECOSAN, 2011). Other commonly consumed foods in the Mediterranean diet, and particularly in the Spanish cuisine, are vegetables, such as onions or peppers, which are often subjected to a frying process for further uses as base ingredients or garnish.

Fried food palatability is related to unique sensory characteristics, including brown colour, crunchy texture and other desired flavour and taste, mainly due to Maillard reactions (Rossell, 2001). Frying process induces significant changes in food such as water loss, melanoidins formation, increase of fat amount, and changes in the fatty acid profile due to the mass exchange between frying media and the fat of food (Sanchez-Muniz, Viejo & Medina, 1992; Romero, Cuesta & Sánchez-Muniz, 2000; Miranda et al., 2010). Maillard reaction also induces the formation of volatile compounds that provide the characteristic aroma and flavour of roasted and fried foods. Among them, furan and furanic compounds can significantly contribute to the sensory properties of heat treated foods (Maga, 1979; Anese & Suman, 2013). However, furan is a highly volatile compound, which has been classified as a possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC, 1995). The Joint FAO/WHO Expert Committee on Food Additives estimates that furan exposure through diet is confirmed as a public health problem (JECFA, 2010). Therefore, Food Safety Agencies promote furan data collection in foods (EFSA, 2010; US FDA, 2008).

Coffee (for adults) and commercial baby foods (for infants) have been proposed as the major contributors to furan exposure (Fromberg, Fagt & Granby, 2009). Some authors have studied the risk assessment of furan in these products (Waizenegger et al., 2012; Lachenmeier, Reusch & Kuballa, 2009), however other cooked foods could also contribute in a high extent to furan exposure due to the fact that furan formation can be influenced by the heat treatment conditions (Fromberg et al., 2009). Carbohydrate degradation, pyrolysis of sugars, decomposition of ascorbic acid and oxidation of polyunsaturated fatty acids during heat treatment can promote furan generation (Perez Locas & Yaylayan, 2004; Becalski & Seaman, 2005; Märk, Pollien, Lindinger, Blank & Mark, 2006; Limacher, Kerler, Conde-Petit & Blank, 2007; Limacher Kerler, Davidek, Schmalzried & Blank, 2008; Owczarek-Fendor et al., 2011). Some authors suggest that carbohydrate foods are more prone to the formation of furan, probably due to the Maillard reaction and that the retention of furan in foods is mainly caused by the lipid fraction, especially polyunsaturated fatty acids (Fromberg et al., 2009; Arisseto, Vicente, Ueno, Tfouni & Toledo, 2011). So that, it may be expected that the content of furan in foods subjected to a frying process, especially those rich in carbohydrates, could be high. Nevertheless, an EFSA report highlights that only 8% of the furan data were reported after food preparation and it claims that future testing of furan should preferably analysed both as purchased and as consumed indicating the exact cooking preparation conditions (time, temperature and handling label information) (EFSA, 2010). Therefore, the main aim of this work was to evaluate the furan content in some of the most common Spanish fried foods (AECOSAN, 2011), both bread-coated frozen foods cooked by deep-frying and selected vegetables slowly fried in oil and commonly used as base ingredients for cooking some typical dishes in Spanish cuisine. The use of reheated oil for frying is also a common culinary practice. Thus, furan occurrence in foods fried with fresh or reheated oils was also tested. With the obtained results, a preliminary approach for risk assessment of furan in fried foods for Spanish population has been conducted.

2. Material and methods

2.1 Food samples and reagents

Three packages of different lots of frozen precooked foods (ham croquettes, squid rings, tuna pasties, churros, nuggets, fish fingers, onion rings and san jacobos) were obtained from a local supermarket. According to food labels of frozen precooked products, carbohydrates were the most abundant nutrient (19.4-30.0 g/100g), followed by fat (0.7-16.3 g/100g) and proteins (<10 g/100g). Three different batches of vegetables (yellow onion, green pepper, cardoon, cabbage and chicory), as well as sunflower oil and olive oil (refined and virgin olive oil blend) were obtained from local stores. Furan and d4-furan, as well as sodium chloride were purchased from Sigma-Aldrich Chemical (Steinheim, Germany). The methanol (HPLC grade) was purchased from Panreac (Barcelona, Spain).

2.2 Standard solutions

Stock solution of d4-furan was prepared by adding 25 μL of d4-furan to 10 mL of methanol in a vial. A 2.5 μg mL^{-1} water working solution was prepared daily. Stock and working solutions of furan were prepared using the same procedure for d4-furan. Then, six calibration standard solutions at concentration ranging from 0.001 to 0.02 μg mL^{-1} were prepared by adding the appropriate amount of furan water working solution (2.5 μg mL^{-1}) into a 20 mL vial containing 3 g of NaCl and 5 mL of deionized water. In addition, 40 μL of the d4-furan water working solution (2.5 μg mL^{-1}) was added to each calibration solution as internal standard.

2.3 Food samples preparation

Eight frozen precooked foods (ham croquettes, squid rings, tuna pasties, churros, nuggets, fish fingers, onion rings and san jacobos (ham and cheese in breadcrumbs)) were deep fried in olive oil using a domestic deep fryer Princess 180710 (DOSEFES S.A., Barcelona, Spain) at 190 ºC during 6 minutes. Temperature was checked with a digital thermometer type J/K Fluke 51 (Fluke, USA). In order to study the effect of the reuse of oil in the formation of furan, 20 times reheated olive oil was also employed. Oil polar compounds were measured with a quality-meter frying oil FOM-320 (Ebro Electronic, Ingolstadt, Germany).

Chopped vegetables (yellow onion, green pepper, cardoon, cabbage and chicory) (300 g) were fried with olive or sunflower oils (30 mL) at 115 ºC for 10 minutes in a frying pan. Then, temperature was decreased to 108 ºC for 5 minutes. Chopped vegetables were also submitted to heating at 150 ºC for 10 minutes and then at 110 ºC for 5 minutes in a griddle without oil addition.

2.4 Furan analysis

Furan content was analysed following the method described by Perez-Palacios, Petisca, Melo & Ferreira (2012) with modifications. Samples were grinded with a fork until homogenization in an ice bath to avoid furan losses. Immediately after, 2 g of each sample were transferred to a 20 mL vial containing 3 g of NaCl and 5 mL of deionized water. For the oil samples, 2 mL of oil were transferred to the vial containing 3 g of NaCl. A volume of 40 µL of working solution of d4-furan (2.5 µg mL^{-1}) was added as internal standard to each vial which was immediately closed. Afterwards, the vial was sonicated for 15 min and stored at 4 °C until further analysis (< 24h). Each sample was prepared in triplicate.

A SPME fiber (Supelco Co., Canada) coated with carboxen/polydimethylxilosane (CAR/PDMS) 75 µm was used. The fiber was exposed to the headspace of the sample during 40 min at 37 °C. The SPME fiber was desorbed at 280 °C for 10 min in a HP6890 GC System gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled to a mass selective detector (MS) (model 5973, Agilent Technologies). Volatiles were separated using a column HP PLOT/Q (30 m length x 0.32 mm internal diameter x 0.20 µm thickness). The carrier gas was helium at a flow of 1 mL min^{-1}. The temperature program was 40 °C for 5 min, then raised at 3 °C min^{-1} to 120 °C, and finally, raised at 10ºC min^{-1} up to 220ºC and held for 5 min. The GC-MS transfer line temperature was 270 °C. The MS operated in the electron impact mode with an electron impact energy of 70 eV and a multiplier voltage of 1247 V and collected data at a rate of 1 scan s^{-1} over a range of m/z 35-350. Ion source temperature was set at 230 °C. Furan and d4-furan were identified by comparing their retention time and their mass spectra with those of standard compounds and NIST 05L library. Selected ion monitoring (SIM) was used for the detection of furan and d4-furan, using m/z 68 and m/z 72, respectively. Furan was quantified using d4-furan as an internal standard by calibrate curve method.

The method was validated by obtaining a linear relationship between the concentrations of furan and the respective area ratio between m/z 68 and m/z 72 (r = 0.999). Results for repeatability showed a good precision of the method with coefficient of variation values below 5%. Taking into account that furan is a highly volatile compound, a narrow dispersion of values was also observed for intermediate precision, with coefficients of variation between 3.28 and 16.19%. The limit of detection (LOD) and limit of quantification (LOQ) were also calculated, obtaining 0.7 and 2.3 μg furan kg^{-1} sample, respectively.

2.5 Estimation of daily furan intake and Margin of Exposure (MOE)

The daily furan exposure in Spanish population due to the analysed fried foods was calculated based on the obtained furan results in the present work and the Spanish Dietary Intake Survey (AECOSAN, 2011) following the next equation:

$$E = \sum (F \times C)$$

Where E is the amount of furan to which a person is exposed (ng/kg bw/day) due to the selected foods; F is the food intake per day (g/kg bw/day or mL/kg bw/day) according to the Spanish dietary consumption survey (AECOSAN, 2011); and C is furan content (μg kg^{-1}) of each food from the present data or those reported by EFSA (2011). Moreover, in order to provide different exposure scenarios and to consider highly exposed individuals the following calculations were carried out: (1) for food intake, the mean and 99th percentile (P99), both according to the Spanish dietary consumption survey (AECOSAN, 2011) were considered for calculations; and (2) for furan content, the mean and 95th percentile (P95) from our samples or those reported by EFSA (2011) were considered for calculations. In analysed food samples where the value is below the LOD or LOQ of the method and to get a range, values were set as "0" for the lower value and LOD or LOQ for the upper bound, respectively.

Finally, as a first approach for risk assessment, the margin of exposure (MOE) was calculated according to the harmonised approach of the European Food Safety Authority (EFSA) for the risk assessment of substances which are genotoxic and carcinogenic (EFSA 2005). The MOE is obtained by dividing the value of the selected reference point on the dose-response curve for the adverse effect of the substance, such as Benchmark Dose Lower Confidence Limit of 10% (BMDL10), by the estimated human intake of the substance. The BMDL10 used for our calculations was 0.96 mg/kg bw/day for induction of hepatocellular adenomas and carcinomas in female mice which is the one used in the last WHO Expert Committee on Food Additives for Furan (WHO, 2011). For the MOE calculation, the estimated intake was calculated taking into account all the fried samples (both with fresh and reheated olive oil) for each frozen precooked food assuming that population is normally exposed to both kinds of frying processes.

2.6 Statistical Analysis

Results are shown as means ± standard deviations. One-way analysis of variance (ANOVA) was applied for each parameter. A Tukey test was applied as *a posteriori* test with a level of significance of 95%. All statistical analyses were performed using the STATA v.12.0 software package.

3. Results and discussion

3.1. Furan in cooked foods

Frying process is a common culinary technique applied to foods as different as bread-coated frozen foods and vegetables to develop their typical sensorial properties or to be used as base ingredients in Spanish cuisine. In this work, the occurrence of furan was tested (1) in eight bread-coated frozen foods selected among the most common consumed in Spain (AECOSAN, 2011) before and after deep frying with fresh or reheated olive oil, and (2) in five vegetables used as base ingredients or garnish in typical dishes in Spanish cuisine after frying with olive or sunflower oil, or after griddling.

Figure 1 shows the presence of furan in the bread-coated frozen foods (ham croquettes, squid rings, tuna pasties, churros, nuggets, fish fingers, onion rings and san jacobos) analysed before and after deep frying. In raw foods, furan was only found in tuna pasties (16 µg kg^{-1}), probably due to their filling (tuna in tomato sauce) previously cooked. Recently, a study on furan occurrence in canned fish found the maximum furan content in those samples containing tomato sauce, including tuna (27 µg kg^{-1}) (Pye & Crews, 2014). In the present samples, the pastry that involves the filling of tuna in tomato sauce, together with the chilling temperature might prevent furan volatilization during food preservation. The low furan values found in fresh olive oil (2.5 µg kg^{-1}) (Table 1) suggest that oil will negligibly contribute to furan content in fried foods. Similarly, Fromberg et al. (2009) and EFSA (EFSA, 2010; EFSA, 2011) showed that vegetable fats hardly ever contribute to furan content, except in the case of olive oil (5.1 µg kg^{-1}) used as frying agent of homemade meat and fish balls (Fromberg et al., 2009). The low furan content in olive oil used in the present study might be related with its freshness (3% polar compounds), which is unknown for olive oil previously reported.

After frying, furan levels increased in all samples. In the case of samples fried in fresh oil, furan levels were ranged between 16 µg kg^{-1} (fish fingers) and 115 µg kg^{-1} (onion rings). When reheated oil was used, furan levels tended to be higher in many cases, ranging from 12 µg kg^{-1} (tuna pasties) and 172 µg kg^{-1} (onion rings). However, no significant differences were found in ham croquettes, tuna pasties, nuggets and fish fingers that were fried using either fresh or reheated oil. Therefore, the use of reheated oil seemed to have lower influence in furan formation than frying process itself. The highest furan levels were observed in fried onion rings, followed by nuggets and churros, while the lowest levels were obtained in some foods containing fish (fish fingers and tuna pasties). Because in the present study the same time (6 min) and temperature (190 ºC) were applied for frying all samples, the different food composition might explain the different levels of furan. The longer time in comparison to that applied for French fries and homemade crisps (3-3.5min at 190 ºC) (Fromberg et al., 2009) might explain the higher values of furan in the present study. Moreover, the Maillard browing reactions induced by frying at high temperatures a dough (churros and tuna pasties) or food samples with a carbohydrate-rich external coat, such as bread (ham croquettes, fish fingers, nuggets and san jacobos) or a dough (squid rings and onion rings), might explain the formation of higher furan content in our samples than in toasted bread slices or also in coffee brew (Fromberg et al, 2009; EFSA, 2011).

Furan occurrence was also measured in olive oil used as frying agent, which was selected because it is the most common vegetable oil used for frying by Spanish population (Sayon-Orea et al., 2014). The results are shown in Table 1. Unheated olive oil contained low amount of furan (2.5 μg kg^{-1}), in agreement with that found in other vegetable oils (EFSA, 2010; EFSA, 2011), but lower than that reported for olive oil by Fromberg et al. (2009). However, olive oil after used as frying agent contained between 14 and 17 μg kg^{-1}. This may be due to the formation of furan from unsaturated fatty acids at high temperatures (Becalski & Seaman, 2005), and the retention of furan formed in fried foods or in their residues by oil (Van Lancker, Adams, Owczarek, De Meulenaer & De Kimpe, 2009). Table 1 also shows the amount of polar compounds before and after frying. Polar compounds significantly increased when the oil was used as frying agent, being higher than the legal limit (25%) (BOE, 1989) in the case of reheated oil after 20 cycles. This indicates thermal oxidation and polymerisation processes that could also promote the formation of furan, not only in the oil, but also in the fried foods, as it was noted above when reheated oil was used.

Table 2 shows the occurrence of furan in five vegetables (yellow onion, green pepper, cardoon, cabbage and chicory) used as base ingredients or garnish in typical dishes in Spanish cuisine, after frying with olive or sunflower oils, or after griddling (heat treatment without oil addition). Furan was only found in griddled onion at low level (3.5 μg kg^{-1}), and in the other griddled vegetables below the limit of quantification of 2.3 μg kg^{-1} or the limit of detection of 0.7 μg kg^{-1}. Furan was not detected in any of the raw or fried vegetable samples. The lower temperature applied (< 115ºC) in comparison to that of the deep-fried foods (190 ºC), the furan volatilization during longer time cooking (15 min vs 6 min) together with the food composition (lower carbohydrates and fat) might explain the low furan content.

3.2. Furan exposure in Spanish population

The estimation of furan potential intake by Spanish population due to some of the most commonly consumed fried foods was made based on the furan results obtained in the present work (mean and P95) and the mean and P99 consumption of the analysed foods reported by the Spanish Dietary Intake Survey (AECOSAN, 2011). When consumption data were not available in the dietary survey, such as in squid rings, onion rings and san jacobos, the mean consumption value for fried foods analysed in the present work was used for furan estimation.

Table 3 and 4 shows the average consumption of the different foods analysed in the present study by the Spanish population (AECOSAN, 2011) and the furan exposure due to these foods. Olive oil is one of the main foods which might contribute to furan intake (up to 1463 ng/ kg bw/day for the worst scenario) due to its high consumption in Spain. However, it has to be considered that most of the olive oil is consumed unheated, mainly in salads, which present very low furan content (2.5 μg kg^{-1}) providing a furan intake of 57 and 192 ng/kg bw/day for those consumers with a mean and P99 food consumption, respectively. Similarly, the high consumption of onion might contribute up to 368 ng/kg bw/day of furan intake for the worst scenario. However, onion is mainly consumed raw in salads or fried, in which samples furan content was not found. Therefore, only in the case of the consumption of onion treated by griddling, which is less common than the other culinary techniques, it contributes to furan dietary exposure. Among fried frozen precooked foods, the highest furan intake is due to onion rings (1.4-54 ng/kg bw/day) and churros (1.3-42 ng/kg bw/day). A total dietary exposure ranged from 4.7 to 178 ng/kg bw/day of furan was estimated for Spanish population due to the consumption of the fried frozen precooked foods analysed in the present study.

The total exposure of furan in Spanish population (Table 5) was also estimated based on data of the furan content (mean and P95) per main food category reported by EFSA (2011) and the mean and P99 consumption of these foods by Spanish population (AECOSAN, 2011). Coffee brew, with a range of furan levels between 42-45 μg kg^{-1} (mean) and 228 μg kg^{-1} (P95) (EFSA, 2011) has proposed as the major contributor to furan intake in European adult population (Fromberg, 2009; EFSA 2011; Sijia, Enting & Yuan, 2014). As EFSA suggested, these values might be overestimated, due to the high consumption of coffee brew in Northern European countries (more than 10 ml/kg bw/day of coffee) that usually has been taken to estimate furan content (Fromberg et al., 2009). In Spain, coffee brew consumption is much lower (up to 5.4 ml/kg bw/day) (AECOSAN, 2011), however coffee is also shown as the main contributor to furan exposure, except for those coffee drinkers with the mean consumption of coffee brews with a mean furan content. After coffee, the highest furan exposure in the Spanish population is due to cereal products. This could be due to the fact that the category “cereal products” in Spanish survey covers a wide range of products, and consequently its consumption might be overestimated. EFSA report only collected furan data from 4 cereal products samples from Spain, but it is difficult to know what are the specific foods included. Taking into account that typical Spanish fried foods such as churros, croquettes and pasties are included in the category “cereal products” (AECOSAN, 2011), and that as far as we know this is the first study who reports furan content in these products, EFSA data should be revised to include them. Regarding vegetables results, furan values reported by EFSA (2011) were higher than those resulting on the present study where furan was scarcely detected. Since canned and jarred products present high levels of furan (US FDA, 2004) it is probably that EFSA data mainly include this kind of vegetables.

A total mean dietary exposure of furan of 239 ng/kg bw/day was estimated for Spanish population. This result is in the range reported by EFSA (2011) for European adults (30 and 580 ng/kg bw/day). However, taking into account the worst scenario, that is the high consumption (P99) of different foods with the highest content of furan (P95), total dietary exposure of furan could reach up to 4372 ng/kg bw/day.

Additionally, to have a preliminary approach for risk assessment, MOEs of furan for fried frozen precooked food samples and for the main food categories established by EFSA, are shown in Tables 4 and 5. According to the EFSA (2005), a MOE of 10,000 or higher would be considered as a low public health concern and reasonably as a low priority for risk management actions. WHO (2011) obtained MOEs of furan of 960 and 480 for average and high dietary exposures, respectively. Therefore, the Committee considered that these MOEs indicate a human health concern for furan. In the case of the total furan exposure in Spanish population (Table 5), MOEs were also below 10,000 in all scenarios. When MOEs were calculated for fried frozen precooked food samples for Spanish population (Table 4), results showed that furan could suppose a possible public health risk only in the case of people with the highest consumption of these food products.

In summary, deep-frying process at high temperatures induces the formation of considerable amounts of furan in bread-coated foods with a furan increase tendency when reheated oil is used, suggesting a health risk in populations groups with a high consumption of these frozen precooked products. Nevertheless, taking into account the limitation of the low number of samples in the present study, as well as the increment of the consumption of these fried products by Spanish population, further studies should be developed to provide exposure data for a final risk assessment of fried foods. Therefore, a higher number of samples from different commercial brands, homemade products, and other ethnic foods should be analysed. Moreover, further studies about the occurrence of furan in fried bread-coated foods should be developed considering other aspects, such as the volatilization of furan during the time between cooking and consumption, due to the fact that some authors found a decrease in furan levels (Zoller, Sager & Reinhard 2007; EFSA, 2011), while others reported a furan increase during cooling in toasted bread (Fromberg et al., 2009). Taking into account that 93.6% of the collected furan results in EFSA report (2011) were derived from samples without cooking processes, data should be continuously revised including higher numbers of foods cooked at different conditions. Furthermore, risk assessment of foods should be conducted to consider vulnerable groups, like adolescents and infants, and professionals in restaurants, caterings, etc.

Acknowledgments

We thank the Spanish Ministry of Economy and Competitiveness (AGL2014-52636-P) and PIUNA (Plan de Investigación de la Universidad de Navarra) for their contribution to the financial support of this work. We also thank Dr. Vettorazzi for her kind assistant and advices in toxicological aspects, and Ms. Gwenaelle Ceniceros for her kind help. I.J wishes to express her gratitude to the Association of Friends of the University of Navarra for the grant received.

The authors declare no conflicts of interest

References

AECOSAN (Agencia Española de Consumo, Seguridad Alimentaria y Nutrición). (2011). ENIDE: Encuesta nacional de ingesta dietética (2009-2010). Resultados sobre datos de consumo. URL http://www.aesan.mspsi.gob.es/AESAN/docs/docs/evaluacion_riesgos/datos_consumo/ENIDE.pdf. Accessed 28.09.15

Anese, M., & Suman, M. (2013). Mitigation strategies of furan and 5-hydroxymethylfurfural in food. *Food Research International, 51*(1), 257-264.

Arisseto A.P., Vicente, E., Ueno, M.S., Tfouni, S.A., & Toledo M.C. (2011) Furan levels in coffee as influenced by species, roast degree, and brewing procedures *Journal of Agricultural and Food Chemistry*, 59, 3118–3124.

Becalski, A., & Seaman, S. (2005). Furan precursors in food: A model study and development of a simple headspace method for determination of furan. *Journal of AOAC International, 88*(1), 102-106.

BOE (Boletín Oficial del Estado). (1989). Orden de 26 de enero de 1989 por la que se aprueba la norma de calidad para los aceites y grasas calentados. URL:http://www.boe.es/diario_boe/txt.php?id=BOE-A-1989-2265. Accessed 28.09.15

EFSA (European Food Safety Authority). (2005). Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. *EFSA Journal, (282) 1-31* URL:http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/282.pdf. Accessed 15.12.15

EFSA (European Food Safety Authority). (2010). Update of results on the monitoring of furan levels in food. *EFSA Journal, 8(7) 1702* URL: http://www.efsa.europa.eu/it/search/doc/1702.pdf. Accessed 28.09.15

EFSA (European Food Safety Authority). (2011). Update on furan levels in food from monitoring years 2004-2010 and exposure assessment. *EFSA Journal, 9 (9):2347* URL: http://www.efsa.europa.eu/en/efsajournal/doc/2347.pdf. Accessed 28.09.15

Fromberg, A.; Fagt, S. & Granby, K. (2009). Furan in heat processed food products including home cooked food products and ready-to-eat products. *EFSA External Scientific Report* URL: http://www.efsa.europa.eu/en/supporting/doc/1e.pdf. Accessed 28.09.15

IARC (International Agency for Research on Cancer). (1995). Furan. *IARC Monographs on the evaluation of carcinogenic risks to humans*, 63, 393-407

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2010). Summary and conclusions of the 72nd JECFA meeting. URL http://www.who.int/foodsafety/chem/summary72_rev.pdf Accessed 28.09.15

Lachenmeier, D.W., Reusch, H. & Kuballa, T. (2009) Risk assessment of furan in commercially jarred baby foods, including insights into its occurrence and formation in freshly home-cooked foods for infants and young children, *Food Additives & Contaminants: Part A*, 26:6, 776-785.

Limacher, A., Kerler, J., Conde-Petit, B., & Blank, I. (2007). Formation of furan and methylfuran from ascorbic acid in model systems and food. *Food Additives & Contaminants, 24*, 122-135.

Limacher, A., Kerler, J., Davidek, T., Schmalzried, F., & Blank, I. (2008). Formation of furan and methylfuran by maillard-type reactions in model systems and food. *Journal of Agricultural and Food Chemistry, 56*(10), 3639-3647.

Maga, J. A. (1979). Furans in foods. *Critical Reviews in Food Science and Nutrition, 11*(4), 355.

MAGRAMA (Ministry of Agriculture, Food and Environment). (2006). La alimentación en España- 2006. Capítulo III: La alimentación española en 2006. URL http://www.magrama.gob.es/en/alimentacion/publicaciones/libro2010-11-04_18.56.25.7562.aspx Accessed 28.09.15

MAGRAMA (Ministry of Agriculture, Food and Environment). (2014). La alimentación mes a mes en España, Agosto 2014 URL http://publicacionesoficiales.boe.es/detail.php?id=630928014-0008. Accessed 28.09.15

Märk, J., Pollien, P., Lindinger, C., Blank, I., & Mark, T. (2006). Quantitation of furan and methylfuran formed in different precursor systems by proton transfer reaction mass spectrometry. *Journal of Agricultural and Food Chemistry, 54*(7), 2786-2793.

Miranda, J. M., Martínez, B., Pérez, B., Antón, X., Vázquez, B. I., Fente, C. A., et al. (2010). The effects of industrial pre-frying and domestic cooking methods on the nutritional compositions and fatty acid profiles of two different frozen breaded foods. *LWT - Food Science and Technology, 43*(8), 1271-1276.

Owczarek-Fendor, A., De Meulenaer, B., Scholl, G., Adams, A., Van Lancker, F., Eppe, G., et al. (2011). Furan formation from lipids in starch-based model systems, as influenced by interactions with antioxidants and proteins. *Journal of Agricultural and Food Chemistry, 59*(6), 2368-2376.

Perez Locas, C., & Yaylayan, V. A. (2004). Origin and mechanistic pathways of formation of the parent Furan. A food toxicant. *Journal of Agricultural and Food Chemistry, 52*(22), 6830-6836.

Perez-Palacios, T., Petisca, C., Melo, A., & Ferreira, I.M.P.L.V.O. (2012). Quantification of furanic compounds in coated deep-fried products simulating normal preparation and consumption: Optimisation of HS-SPME analytical conditions by response surface methodology. *Food Chemistry, 135*(3), 1337-1343.

Pye, C., & Crews, C. (2014). Furan in canned sardines and other fish. *Food Additives & Contaminants: Part B, 7*(1), 43-45.

Romero, A., Cuesta, C., & Sanchez-Muniz, F. (2000). Cyclic fatty acid monomers and thermoxidative alteration compounds formed during frying of frozen foods in extra virgin olive oil. Journal of the American Oil Chemists' Society, 77(11), 1169-1175.

Rossell, J.B., 2001. Frying: Improving Quality. *Woodhead Publishing Limited, Cambridge*, 1–355.

Sanchez-Muniz, F., Viejo, J. M., & Medina, R. (1992). Deep-frying of sardines in different culinary fats. changes in the fatty acid composition of sardines and frying fats. *Journal of Agricultural and Food Chemistry, 40*(11), 2252-2256.

Sayon-Orea, C., Martinez-Gonzalez, M. A., Gea, A., Flores-Gomez, E., Basterra-Gortari, F. J., & Bes-Rastrollo, M. (2014). Consumption of fried foods and risk of metabolic syndrome: The SUN cohort study. *Clinical Nutrition, 33*(3), 545-549.

Sijia, W., Enting, W., Yuan, Y. (2014). Detection of furan levels in selected Chinese foods by solid phase microextraction-gas chromatography/mass spectrometry method and dietary exposure estimation of furan in the Chinese population. *Food and Chemical Toxicology*, 64, 34-40.

US FDA (United States Food and Drug Administration). (2004). Exploratory Data on Furan in Food. URL: http://www.fda.gov/ohrms/dockets/ac/04/briefing/4045b2_09_furan%20data.pdf. Accessed 07.01.16

US FDA (United States Food and Drug Administration). (2008). Exploratory data on furan in food—individual food products. URL: http://www.fda.gov/food/foodborneillnesscontaminants/chemicalcontaminants/ucm078439.htm. Accessed 28.09.15

Van Lancker, F., Adams, A., Owczarek, A., De Meulenaer, B., & De Kimpe, N. (2009). Impact of various food ingredients on the retention of furan in foods. *Molecular Nutrition & Food Research*, 53(12), 1505-1511.

Waizenegger, J., Winkler, G., Kuballa, T., Ruge, W., Kersting, M., Alexy, U. & Lachenmeier, D.W. (2012) Analysis and risk assessment of furan in coffee products targeted to adolescents. *Food Additives & Contaminants: Part A*, 29(1), 19-28

WHO (World health organization),(2011). Safety evaluation if certain contaminants in food. *WHO Food Additive series* (63) 487-605. URL: http://apps.who.int/iris/bitstream/10665/44520/1/9789241660631_eng.pdf. Access 15.12.15

Zoller, O., Sager, F., & Reinhard, H. (2007). Furan in food: Headspace method and product survey. *Food Additives and Contaminants, 24*(sup1), 91.

Figure caption

Figure 1. Furan (μg kg^{-1}) in frozen precooked bread-coated foods before and after deep frying treatment with fresh and reheated olive oil

Table 1. Furan levels and polar compounds in olive oil before frying (unheated), and after one (heated) or 20 frying cycles (reheated).

	Unheated olive oil	Heated olive oil (1 frying cycle)	Reheated olive oil (20 frying cycles)
Furan (µg kg^{-1})	2.5 ± 0.2 ^{b}	17 ± 3 ^{a}	14 ± 3 ^{a}
Polar compounds (%)	3.0 ± 0.0 ^{c}	20.3 ± 1.0 ^{b}	34.5 ± 1.8 ^{a}

Values are shown as means ± standard deviations (n=3).
Different letters for row indicate significant differences.

Table 2. Furan (μg kg^{-1}) in vegetables before and after heat treatment

Vegetable	Heat treatment	Furan (μg kg^{-1})
Onion	Raw	nd
	Fried in olive oil	nd
	Fried in sunflower oil	nd
	Griddled	3.5 ± 0.3
Green pepper	Raw	nd
	Fried in olive oil	nd
	Fried in sunflower oil	nd
	Griddled	nd
Cardoon	Raw	nd
	Fried in olive oil	nd
	Fried in sunflower oil	nd
	Griddled	<2.3
Cabbage	Raw	nd
	Fried in olive oil	nd
	Fried in sunflower oil	nd
	Griddled	<2.3
Chicory	Raw	nd
	Fried in olive oil	nd
	Fried in sunflower oil	nd
	Griddled	<0.7

Values are shown as means ± standard deviations (n=3).
nd, not detected.

Table 3. Food consumption and furan levels (ng/kg bw/day) per selected foods in the Spanish population.

Food	**Food consumption**[a] (g/kg bw/day or mL/kg bw/day)		**Furan content**[b] (µg/kg)		
	Mean	P99	Range	Mean	P95
Ham croquettes	0.012	0.47	12 – 32	23	32
Squid rings [c]	0.010	0.30	30 – 90	59	88
Tuna pasties	0.009	0.38	12 – 28	19	28
Churros	0.021	0.53	47 – 81	63	80
Nuggets	0.005	0.077	54 – 86	71	85
Fish fingers	0.002	0.048	7.6 – 23	17	22
Onion rings [c]	0.010	0.30	106 – 181	143	179
San Jacobos [c]	0.010	0.30	31 – 74	51	73
Olive oil	23	77	2.4 – 20	9.7	19
Onion	23	105	0 – 3.5	--	--
Green pepper	14	115	0 – 0.70	--	--
Cardoon	0.16	3.1	0 – 2.3	--	--
Cabbage	2.3	66.7	0 – 2.3	--	--
Chicory	0.010	0.32	0 – 0.70	--	--

[a] Mean and P99 food consumption by Spanish population as recorded by AECOSAN(2011).

[b] Results obtained from samples fried with fresh and reheated olive oil (n=6).[c] Mean values of fried foods consumption are taken because data are not available.

Table 4. Furan exposure (ng/kg bw/day) and Margin of Exposure (MOE) per selected foods in the Spanish population.

Food	**Furan exposure** (ng/kg bw/day)		**MOE** [a]	
	Intake: Mean	Intake: P99	Intake: Mean	Intake: P99
	Furan concentration: Mean / P95	Furan concentration: Mean / P95	Furan concentration: Mean / P95	Furan concentration: Mean / P95
Ham croquettes	0.27 / 0.38	11 / 15	3468459 / 2524854	88181/ 64191
Squid rings	0.59 / 0.88	18/ 27	1629696 / 1086956	54142 / 36111
Tuna pasties	0.17 / 0.25	7.2 / 10	5561831 / 3828667	132775 / 91400
Churros	1.3 / 1.7	33 / 42	722793 / 570750	28802 / 22743
Nuggets	0.35 / 0.42	5.4 / 6.5	2713717 / 2267024	176215 / 147209
Fish fingers	0.030 / 0.040	0.83 / 1.1	27655080 / 21323856	1152295 / 888494
Onion rings	1.4 / 1.8	43/ 54	670071 / 536770	22261 / 17832
San Jacobos	0.50 / 0.73	15/ 22	1898483 / 1312685	63072 / 43610
TOTAL	**4.7 / 6.2**	**134 / 178**	**204540 / 155228**	**7174 / 5402**

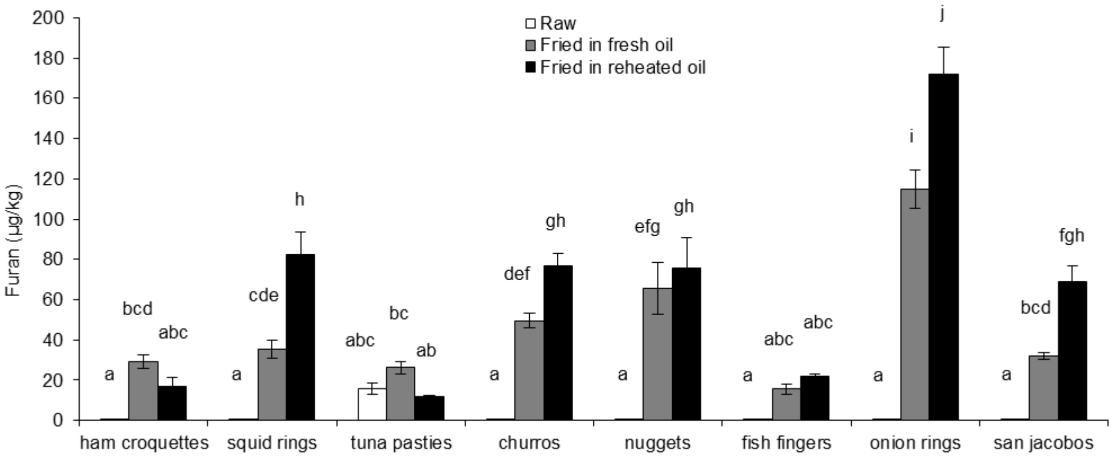
[a] Margin of exposure (MOE) to furan for different exposure scenarios. Calculated with The BMDL10 used in the last WHO Expert Committee on Food Additives for Furan (WHO, 2011) (0.96 mg/kg b.w.)

Table 5. Food consumption, furan content, furan exposure (ng/kg bw/day) and Margin of exposure (MOE) per main food category in the Spanish population.

Food	**Food consumption** [a] (g/kg bw/day or mL/kg bw/day)		**Furan content** [b] (µg/kg)		**Furan exposure** (ng/kg bw/day)		**MOE** [c]	
	Mean	P99	Mean	P95	Intake: Mean Furan concentration: Mean / P95	Intake: P99 Furan concentration: Mean / P95	Intake: Mean Furan concentration: Mean / P95	Intake: P99 Furan concentration: Mean / P95
Coffee brew	0.95	5.4	42 – 45	228	41 / 217	231 / 1227	23500 / 4432	4149 / 782
Baked beans	0.004	0.09	22 – 24	57	0.090 / 0.23	2.1 / 5.1	10666666 / 4173913	463768 / 187134
Beer	1.4	15	3.3 – 5.2	13	6.1 / 19	65 / 199	156862 / 51282	14734 / 4817
Cereal products	3.2	9.2	15 – 18	60	53 / 194	152 / 551	17957 / 4938	6330 / 1741
Fish	1.5	5.4	17	86	15 / 128	81 / 467	64042 / 7491	6979 / 2055
Fruit juices	1.1	12	2.2 – 4.6	8-10	3.9 / 10	41 / 110	245524 / 92753	23162 / 8750
Fruits	3.6	14	2 – 6.4	11	15 / 39	59 / 155	64386 / 24583	16164 / 6172
Meat products	2.7	8.1	13 – 17	67	41 / 183	121 / 542	23443 / 5248	7911 / 1771
Milk products	5.1	13	5 – 5.6	20	27 / 102	69 / 260	35661 / 9448	13933 / 3692
Sauces	0.17	1.4	8.3 – 11	30	1.6 / 5.1	13 / 42	585365 / 188235	71569 / 23021
Soups	0.039	1.4	23 – 24	72	0.92 / 2.8	33 / 100	1043478 / 341637	29389 / 3592
Soy sauce	0.017	0.30	27	67	0.46 / 1.1	8.1 / 20	2086956 / 842105	6971678 / 47761
Vegetables juices	na	Na	2.9 – 9	18	na	na	na	na
Vegetables	2.9	9.7	6.9 – 9.6	41	25 / 122	80 / 399	39056 / 7857	11959 / 2406
Cocoa	0.050	0.78	9 – 10	40	0.47 / 2.0	7.4 / 31	2042553 / 888888	129554 / 30769
Snacks and crisps	0.040	0.72	9.6 – 10	27	0.39 / 1.1	7.1/ 19	461538 / 102564	136054 / 49382
Soft drinks	2.1	17	0.8 – 1.2	4.5	2.1 / 9.4	17 / 79	461538 / 102564	54794 / 12176
Soya products	na	na	6.7	28	na	na	na	na
Sweets	0.24	1.3	5 – 6	18	1.3 / 4.3	7.2 / 24	727272 / 222222	133240 / 40712
Tea	0.51	6.7	1 – 1.7	3.3	0.69 / 1.7	8.9 / 22	1391304 / 571428	106773 / 43680
Vegetables fat	0.53	1.4	1.5 – 1.7	10	0.85 / 5.3	2.2 / 14	1129411 / 181132	431654 / 69064
Wine and liquors	2.4	19	1.3	5.6	3.2 / 14	24 / 105	302839 / 70278	16081 / 9108
Total					**239 / 1061**	**1032 / 4372**	**4021 / 905**	**930 / 220**

[a] Mean and P99 food consumption by Spanish population as recorded by AECOSAN (2011). [b] Mean furan content in food per main food category by EFSA (2011). [c] Margin of exposure (MOE) to furan for different exposure scenarios. Calculated with The BMDL10 used in the last WHO Expert Committee on Food Additives for Furan (WHO, 2011) (0.96 mg/kg b.w.) na. Not data available.

Figure 1. Furan (µg kg^{-1}) in frozen precooked bread-coated foods before and after deep frying treatment with fresh and reheated olive oil



Different letters indicate significant differences ($p \leq 0.05$)
Results are shown as means ± standard deviations (n=3)