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### Highlights

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- ▶ ~~It is firstly evaluated the influence of cooking and handling conditions on furanic compounds levels in breaded fish products.~~
  - ▶ ~~The generation of furanic compounds could be minimized by adjusting the cooking method and conditions.~~ ▶ ~~Oven-baking and reheating in the microwave are preferred than deep frying for reducing the generation of furanic compounds.~~ ▶ ~~Oven-baking and reheating in the microwave also reduce volatile compounds related to the aroma and flavour of fried food.~~ ▶ ~~It should be achieved cooking conditions that~~
  - Q2 ~~reduce the formation of furanic compounds without influencing volatile profile.~~
-



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# Food and Chemical Toxicology

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## Impact of cooking and handling conditions on furanic compounds in breaded fish products

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### ABSTRACT

This study evaluates the influence of cooking and handling conditions on the quantity of furanic compounds (furan, 2-furfural, furfuryl alcohol, 2-pentylfuran, 5-hydroxymethylfurfural) in breaded fish products. Oven-baking and reheating in the microwave lead to low furanic compounds formation in comparison with deep-frying. The use of olive oil for deep-frying promoted higher levels of furanic compounds than sunflower oil. The amounts of these compounds diminished as the temperature and time of deep-frying decreased as well as after a delay after deep-frying. Thus, the generation of furanic compounds can be minimized by adjusting the cooking method and conditions, such as using an electric oven, deep-frying in sunflower oil at 160 °C during 4 min, or waiting 10 min after cooking. However, these conditions that reduce furanic compounds levels also reduce the content of volatile compounds related to the aroma and flavour of fried foods. In this sense, new efforts should be done to reduce the formation of furanic compound without being detrimental to the volatile profile.

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### 1. Introduction

The development of aroma and flavour in cooked products is a complex process in which different compounds react to produce intermediary or volatile compounds. Frying induces oxidation and Maillard reactions, which are essential for the final aroma and flavour attributes of the food (Bastida and Sanchez-Muniz, 2001; Romero et al., 2000) and simultaneously responsible for the formation of undesirable and compounds (Mottram, 1998; Nawar, 1998).

Furanic compounds are recognized as important contributors to the characteristic odour of fried products (Wagner and Grosch, 1998; Cerny and Grosch, 1992), particularly in coated products, due to the intense heat effect on carbohydrates and polyunsaturated fatty acids. These compounds have low thresholds and provide pleasant odour characteristic, such as cocoa, butter or fruity (Belitz and Grosch, 1997). However, furan is considered a

possible human carcinogen (Group 2B) by the International Agency for Research on Cancer (IARC, 1995). Furan can be formed from various precursors naturally present in foods, namely, ascorbic acid, carbohydrates, amino acids, fatty acids, and carotenoids (Pérez-Locas and Yaylayan, 2004; Becalski and Seaman, 2005; Mark et al., 2006; Limacher et al., 2007, 2008; Fan et al., 2008). Most of the results concerning furan content in foods (coffee, infant formula, baby food, cereal, meat, fish, vegetal, dairy and fruit products) have been obtained from samples analysed as purchased, however, it is claimed that further evaluation of furan content in foods should be performed in the samples as they are consumed (EFSA, 2010). Thus, the effect of usual cooking conditions, namely time, temperature and handling information should be studied. Additionally, furan derivatives should also be analysed since they are also present in thermally treated products, for example 2-furfural, furfuryl alcohol and 2-pentylfuran were found in smoked-cured bacon by Yu et al. (2008). Gili et al. (2010) found 2-ethylfuran, 2-butylfuran, 2-acetylfuran, 2-pentylfuran, 2-furfural and furfuryl alcohol in paste fish. There are also studies revealing the toxicity of these furanic compounds in animals and humans (Sujatha, 2008; Arts et al., 2004; Goldsworthy et al., 2001; Wilson et al., 1992).

Concerning 5-hydroxymethylfurfural (HMF), it is less volatile than other furanic compounds and has been mainly analysed in cereal products, honey, fruit products and coffee (Capuano and Fogliano, 2011; Abraham et al., 2011). This furanic compound is an intermediate product in the Maillard reaction (Berg and Van Boekel, 1994; Morales et al., 1997) and is also formed from the

*Abbreviations:* MO, frozen breaded fish products deep-fried in sunflower oil and reheated in the microwave; OL, frozen breaded fish products deep-fried in olive oil; OV, frozen breaded fish products oven-baked; SF, frozen breaded fish products deep-fried in sunflower oil; STO, frozen breaded fish products deep-fried in sunflower oil and analysed straightaway; ST1, frozen breaded fish products deep-fried in sunflower oil and analysed with a delay of 10 min; ST2, frozen breaded fish products deep-fried in sunflower oil and analysed with a delay of 20 min.

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degradation of sugars at high temperatures (Kroh, 1994). HMF is usually used to evaluate the quality of processing and it also raises toxicological concerns (Capuano and Fogliano, 2011; Abraham et al., 2011).

The influence of the various factors involved in a culinary process on the nutritive value of processed foods is of major concern (Guidurus et al., 2010). In house holding conditions the controllable variables are the cooking process, the oil variety and time/temperature of processing. Several studies about the modifications of fat and oil composition during heating and frying under very different conditions have been carried out (Dobarganes et al., 2000). However, papers on the changes in the foods are less abundant, and most of them focused on fat uptake and water loss mechanisms during frying (Debnath et al., 2003). Moreira et al. (1997) studied the effect of oil temperature (130, 160 and 190 °C) on the final oil content of tortilla chips. Miranda et al. (2010) evaluated how the cooking method (baking and deep frying in olive or sunflower oil) affects fatty acids profiles of frozen breaded foods.

Recent results obtained for furanic compounds in coated deep-fried fish have indicated that this type of product should be included in the group of food products with high furan content, such as coffee samples (Pérez-Palacios et al., 2012a). Thus, this work aims to study the effect of cooking and handling conditions (deep-frying at different combinations of time and temperature; deep-frying in different oils and dry oven-baking; reheated in the microwave oven; time after cooking) on the content of furanic compound in a breaded fish product, in order to provide data on the reduction of these compounds. Moreover, the influence of the cooking conditions on the profile of volatile compounds was also investigated. Coated fish products are very consumed by kids and teenagers due to its high sensory acceptance, its assumed good health characteristics (i.e. high polyunsaturated fatty acid content) and its quick and easy preparation.

## 2. Materials and methods

### 2.1. Chemical and standards

D<sub>4</sub>-furan (98%) was provided by ISOTEC (Ohio, USA). Furan (≥99%), furfuryl alcohol (99%) were supplied by SIGMA ALDRICH (Steinheim, Germany). Furfural was purchased by MERCK (99%) (Darmstadt, Germany). 2-pentylfuran (98%) was provided by ALFAAESAR (Karlsruhe, Germany). HMF (98%) was supplied by Sigma-Aldrich (Steinheim, Germany). Ethyl acetate, hexane, sodium formate, formic acid, and methanol were supplied by Merck (Darmstadt, Germany), and ultrapure water (0.055 µS/cm) was obtained by using a Serial Milli-Q system for Millipore (Supor DCF, Gelman Sciences, Chentelham, Australia). Frozen breaded fish products, sunflower and olive oils (extra virgin with 0.8% acidity) were obtained from a local stored.

### 2.2. Experimental design

Frozen breaded fish products were prepared by deep-frying in sunflower (n = 6) (SF) and olive oil (n = 6) (OL) using a domestic deep-fryer (KENWOOD DF-150; 1 l) at 180 °C during 4 min, and by baking using an electric oven (ELECTRIC Co MF22VD, 22 l), at 200 °C during 17 min (turning over after first nine min) (OV) (n = 6). These

conditions are recommended by the manufacturer. Deep-frying and oven temperature was monitored using a cooking thermometer (Model 26003DeltaTRAK, USA). Deep-frying and oven temperature was monitored using a cooking thermometer (Model 26003DeltaTRAK, USA). In addition, other batch of breaded fish products (n = 6) were deep-fried in sunflower oil (at 180 °C during 4 min), placed into the fridge (4 °C) during 16 h and reheated in a domestic microwave (HAIER M01700, 17 l) at 750 W during 40 s (MO).

Moreover, the breaded fish products were deep-fried in sunflower oil at nine different combinations (n = 6) of temperature (160, 180 and 200 °C) and time (2, 4 and 6 min).

In order to study the stability of the volatile compounds, three batches of breaded fish products were deep-fried in sunflower oil and analysed straightaway (n = 6) (ST0) and with a delay of 10 min (n = 6) (ST1) and 20 min (n = 6) (ST2) kept at ambient temperature (18–20 °C).

All samples were slightly drained after frying, placed on paper towel for removing external excess oil, and grinding by using a device named “masticator shears straight” (BUENO HERMANOS, S.A., La Rioja, Spain, ISO 9001-2000 Quality Certified Company) which simulates the chewing process. The oil was replaced every six fry-ing sessions. All samples were processed individually.

### 2.3. Standard solutions

Stock solution of d<sub>4</sub>-furan 2 mg ml<sup>-1</sup> was prepared by injecting 20 µl of refrigerated d<sub>4</sub>-furan with a syringe through the septum of a 10 ml headspace (HS) vial filled with 10 ml of methanol and sealed. The exact weight of methanol and d<sub>4</sub>-furan was recorded, expressing the concentration in mg ml<sup>-1</sup> and taking into account the density of methanol. Working solutions 1 mg ml<sup>-1</sup> were prepared daily by adding 500 µl of stock solution to a 2 ml vial containing 500 µl of water using the same procedure.

A standard calibration solution containing 8.69, 0.52, 10.84 and 0.07 mg ml<sup>-1</sup> for furan, furfural, furfuryl alcohol and 2-pentylfuran, respectively, was prepared by the addition of 100, 5, 100 and 5 µl of furan, 2-furfural, furfuryl alcohol and 2-pentylfuran, respectively, into a 15 ml HS vial containing 15 ml of methanol. The exact weight of methanol and each added furanic compound was recorded.

Stock solution of HMF in methanol 1.2 mg ml<sup>-1</sup> was prepared. A standard working solution 0.056 mg ml<sup>-1</sup> was made by injecting 50 µl of the stock solution into a vial containing 1 ml of the 40 mM sodium formate (pH = 3)/methanol (1:1) mixture. The exact weight of methanol and HMF was recorded, expressing the concentration in microgram per microlitre. Consecutive dilutions of the standard calibration solution in methanol were made.

### 2.4. Volatile analysis

The procedure used for the quantification of furanic compounds was described by Pérez-Palacios et al. (2012a). Straightaway after grinding, 2 g of sample were transferred to a 50 ml headspace (HS) vial, containing 5 ml of water and 3 g of NaCl. 100 µl of d<sub>4</sub>-furan work solution was added, and the vial immediately sealed at once and kept at -4 °C during 10 min. Afterwards, the vial was placed into an ultrasonic cleaner (FUNILAB, Portugal) during 15 min. To extract furanic compounds a carboxen-polydimethylsiloxane (CAR-PDMS) solid phase microextraction (SPME) fibre (75 µm thickness, Supelco Co., Bellefonte, PA, USA) was used. Prior to analysis, the SPME fibre was preconditioned at 300 °C for 60 min in the chromatograph injection port. The fibre was inserted into the sample vial through the septum and exposed to the HS for 40 min at 37 ± 1 °C under constant agitation (600 rpm). Thereafter, the SPME fibre was inserted and desorbed for 10 min at 280 °C, in the split-less mode, with 1 ml min<sup>-1</sup> flow.

Chromatographic analysis was performed using an Agilent 6890 gas chromatograph (GC) (Agilent, Avondale, PA, USA) coupled to a mass selective (MS) detector (Agilent 5973). Volatiles were separated on a 5% phenyl-methyl silicone (HP-5) bonded phase fused-silica capillary column (Hewlett-Packard, Palo Alto, CA, USA; 60 m × 320 µm i.d., film thickness 1 µm), operating at 80 kPa column head

**Table 1**  
Content of furanic compounds (µg/g sample) in breaded fish products deep-fried in sunflower (SF) and olive (OL) oils, oven-baked (OV) and reheated in the microwave after deep-frying in sunflower oil (MO).

	SF	OL	OV	MO	p
Furan	5.51 ± 0.37 <sup>a</sup>	4.02 ± 0.61 <sup>b</sup>	4.36 ± 0.75 <sup>ab</sup>	ND	<0.001
2-Furfural	0.23 ± 0.01 <sup>b</sup>	0.57 ± 0.01 <sup>a</sup>	ND	0.06 ± 0.02 <sup>c</sup>	<0.001
Furfuryl alcohol	10.54 ± 0.20 <sup>b</sup>	18.87 ± 1.25 <sup>a</sup>	4.67 ± 0.19 <sup>d</sup>	7.13 ± 0.23 <sup>c</sup>	<0.001
2-Pentylfuran	1.50 ± 0.32 <sup>b</sup>	2.23 ± 0.13 <sup>a</sup>	0.25 ± 0.09 <sup>c</sup>	1.09 ± 0.05 <sup>b</sup>	<0.001
HMF	1.78 ± 0.08 <sup>b</sup>	4.47 ± 0.98 <sup>a</sup>	0.64 ± 0.09 <sup>bc</sup>	ND	<0.001
TOTAL	19.46 ± 0.88 <sup>b</sup>	30.36 ± 2.68 <sup>a</sup>	9.99 ± 1.08 <sup>c</sup>	8.15 ± 0.29 <sup>c</sup>	<0.001

On the same row, means with different letters differ significantly (p < 0.05).

ND: nondetected.

\* The results are expressed as means values ± standard deviation.

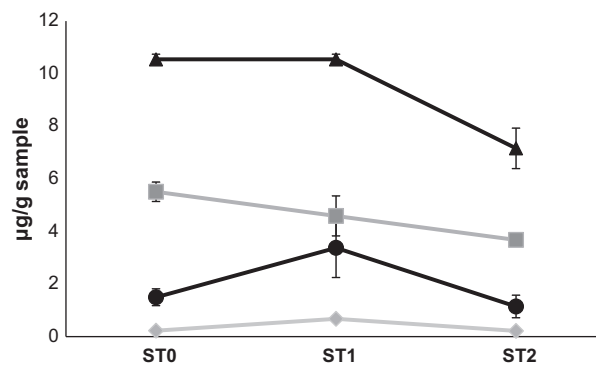
**Table 2**  
Content of furanic compounds ( $\mu\text{g/g}$  sample) in breaded fish products deep-fried in sunflower oil at different combinations of time (t) and temperature ( $T^{\circ}$ ):

	160 °C						180 °C						200 °C						p (t)	p ( $T^{\circ}$ )	p (t- $T^{\circ}$ )
	2 min		4 min		6 min		2 min		4 min		6 min		2 min		4 min		6 min				
Furan	3.64 ± 0.03 <sup>d</sup>	3.78 ± 0.09 <sup>cd</sup>	4.17 ± 0.44 <sup>bcd</sup>	0.14 ± 0.01 <sup>c</sup>	3.76 ± 0.02 <sup>d</sup>	5.51 ± 0.37 <sup>a</sup>	5.08 ± 0.26 <sup>ab</sup>	4.05 ± 0.62 <sup>bcd</sup>	4.94 ± 0.78 <sup>abc</sup>	5.15 ± 0.33 <sup>ab</sup>	4.05 ± 0.62 <sup>bcd</sup>	5.08 ± 0.26 <sup>ab</sup>	4.05 ± 0.62 <sup>bcd</sup>	4.94 ± 0.78 <sup>abc</sup>	5.15 ± 0.33 <sup>ab</sup>	<0.001	0.001	<0.001			
2-furfural	ND	ND	0.14 ± 0.01 <sup>c</sup>	0.14 ± 0.01 <sup>c</sup>	ND	0.23 ± 0.01 <sup>c</sup>	0.64 ± 0.07 <sup>ab</sup>	0.03 ± 0.02 <sup>c</sup>	0.49 ± 0.11 <sup>b</sup>	0.82 ± 0.22 <sup>a</sup>	0.03 ± 0.02 <sup>c</sup>	0.64 ± 0.07 <sup>ab</sup>	0.03 ± 0.02 <sup>c</sup>	0.49 ± 0.11 <sup>b</sup>	0.82 ± 0.22 <sup>a</sup>	<0.001	<0.001	<0.001			
Furfuryl alcohol	4.75 ± 0.40 <sup>e</sup>	4.58 ± 0.17 <sup>e</sup>	7.02 ± 0.85 <sup>de</sup>	7.02 ± 0.85 <sup>de</sup>	5.07 ± 0.46 <sup>e</sup>	10.54 ± 0.20 <sup>cd</sup>	17.30 ± 1.82 <sup>ab</sup>	5.37 ± 0.42 <sup>e</sup>	14.27 ± 0.38 <sup>bc</sup>	20.19 ± 4.55 <sup>a</sup>	5.37 ± 0.42 <sup>e</sup>	17.30 ± 1.82 <sup>ab</sup>	5.37 ± 0.42 <sup>e</sup>	14.27 ± 0.38 <sup>bc</sup>	20.19 ± 4.55 <sup>a</sup>	<0.001	<0.001	<0.001			
2-pentylfuran	1.56 ± 0.08 <sup>e</sup>	2.24 ± 0.35 <sup>bc</sup>	3.88 ± 1.03 <sup>a</sup>	3.88 ± 1.03 <sup>a</sup>	1.81 ± 0.07 <sup>bc</sup>	1.50 ± 0.32 <sup>c</sup>	3.21 ± 0.70 <sup>ab</sup>	2.12 ± 0.41 <sup>bc</sup>	2.15 ± 0.64 <sup>bc</sup>	3.33 ± 0.55 <sup>ab</sup>	2.12 ± 0.41 <sup>bc</sup>	3.21 ± 0.70 <sup>ab</sup>	2.12 ± 0.41 <sup>bc</sup>	3.33 ± 0.55 <sup>ab</sup>	<0.001	0.271	<0.001	<0.001			
HMF	0.82 ± 0.14 <sup>d</sup>	0.96 ± 0.06 <sup>d</sup>	1.76 ± 0.08 <sup>d</sup>	1.76 ± 0.08 <sup>d</sup>	1.34 ± 0.28 <sup>d</sup>	1.78 ± 0.08 <sup>d</sup>	3.61 ± 0.71 <sup>bc</sup>	2.06 ± 0.21 <sup>cd</sup>	3.77 ± 0.17 <sup>b</sup>	9.69 ± 1.50 <sup>a</sup>	2.06 ± 0.21 <sup>cd</sup>	3.61 ± 0.71 <sup>bc</sup>	2.06 ± 0.21 <sup>cd</sup>	3.77 ± 0.17 <sup>b</sup>	<0.001	<0.001	<0.001	<0.001			
Total	10.65 ± 0.53 <sup>e</sup>	11.76 ± 0.75 <sup>e</sup>	16.80 ± 2.59 <sup>cd</sup>	16.80 ± 2.59 <sup>cd</sup>	11.76 ± 0.86 <sup>e</sup>	19.52 ± 0.92 <sup>c</sup>	29.29 ± 3.05 <sup>b</sup>	13.85 ± 1.82 <sup>d</sup>	25.73 ± 1.76 <sup>bc</sup>	38.83 ± 6.32 <sup>a</sup>	13.85 ± 1.82 <sup>d</sup>	29.29 ± 3.05 <sup>b</sup>	13.85 ± 1.82 <sup>d</sup>	25.73 ± 1.76 <sup>bc</sup>	<0.001	<0.001	<0.001	<0.001			

Different letters within the same row differed significantly ( $p < 0.05$ ).

ND: **nondetected**.

\* The results are expressed as means values  $\pm$  standard deviation.



**Fig. 1.** Content of furan (■), 2-furfural (◆), furfuryl alcohol (▲) and 2-pentylfuran (●) in deep-fried breaded fish samples analysed immediately after cooking (ST0) and with a delay of 10 (ST1) and 20 min (ST2).

pressure, resulting in a flow of  $1 \text{ ml min}^{-1}$  at  $40 \text{ }^{\circ}\text{C}$ . The oven temperature programme was isothermal for 5 min at  $40 \text{ }^{\circ}\text{C}$ , raised to  $135 \text{ }^{\circ}\text{C}$  at a rate of  $3 \text{ }^{\circ}\text{C min}^{-1}$  and then raised to  $220 \text{ }^{\circ}\text{C}$  at  $20 \text{ }^{\circ}\text{C min}^{-1}$ . The transfer line to the mass spectrometer was maintained at  $250 \text{ }^{\circ}\text{C}$ . Mass spectra were obtained by electronic impact at  $70 \text{ eV}$ , with a multiplier voltage of  $2056 \text{ V}$ , collecting data at a rate of  $1 \text{ scan s}^{-1}$  over the  $m/z$  range  $30\text{--}500$ . Volatile compounds were identified by comparison of their mass spectrum with reference compounds in the NIST 98 data bank (NIST/EPA/NIST Mass Spectral Library, version 1.6, U.S.A.). Fibre blanks were run daily to ensure the absence of contaminants or carry-over.

Furan and its derivatives were also detected by  $m/z$  characteristic ion, using  $m/z$  68,  $m/z$  72,  $m/z$  96,  $m/z$  98 and  $m/z$  138 for furan,  $d_4$ -furan, 2-furfural, furfuryl alcohol and 2-pentylfuran, respectively. They were quantified by external calibration curve method. Five consecutive dilutions of the standard calibration solution in methanol (1:10, v/v) were prepared.  $100 \mu\text{l}$  of the corresponding standard solution and a fixed volume ( $100 \mu\text{l}$ ) of  $d_4$ -furan working solution were placed in a  $50 \text{ ml}$  HS vial containing  $5 \text{ ml}$  of water and  $3 \text{ g}$  of NaCl, following the same preparation and analysis conditions of samples. For each individual furanic compound a calibration curve (furanic compound peak area/ $d_4$ -furan peak area vs. furanic compound amount/ $d_4$ -furan amount) was constructed, obtaining  $R^2$  values of  $0.9999$ . The final results, expressed in  $\mu\text{g g}^{-1}$ , take into account the exact weight of the sample portion in the HS vial.

## 2.5. HMF analysis

The HMF analysis was done as described by Pérez-Palacios et al. (2012b). Five grams of the grinded coated products was mixed with  $10 \text{ ml}$  ethyl acetate/hexane(4:1, v/v). The mixture was shaken for  $1 \text{ min}$ , centrifuged (5810R Centrifuge, Eppendorf AG, Hamburg, Germany) ( $10 \text{ min}$ ,  $3000 \text{ rpm}$ ), and filtered. This filtrate was mixed with  $550 \mu\text{l}$  of  $40 \text{ mM}$  sodium formate adjusted to  $\text{pH} = 3$  with formic acid (98–100%:methanol 100% (1:1, v/v), and shaken vigorously. The final biphasic system was allowed to separate by centrifugation ( $5 \text{ min}$ ,  $10000 \text{ rpm}$ ). The upper organic phase was eliminated, and the lower aqueous phase ( $300 \mu\text{l}$ ) was used for analysis.

A  $20 \mu\text{l}$  portion of the final extract was injected onto an Ultracarb ODS column ( $5 \mu\text{m}$ ,  $250 \text{ mm}$  length,  $4.6 \text{ mm}$  i.d.) for high performance liquid chromatography (HPLC) – diode array detector (DAD) analysis. It was used an analytical HPLC unit (Jasco, Tokyo, Japan) equipped with Jasco PU-2080HPLC pumps,  $\text{AR}$ , MD-2010 Plus multi wave length detector and a type 7725i Rheodyne injector with a  $20 \mu\text{l}$  loop. Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France) was also used.

Chromatographic separation was performed using a gradient elution of sodium formate  $40 \text{ mM}$  adjusted to  $\text{pH} = 3$  with formic acid (A) and methanol 100% (B). The linear gradient program used was  $t = 0\text{--}5 \text{ min}$ ,  $95\% \text{ A}$ ;  $t = 20 \text{ min}$ ,  $60\% \text{ A}$ ;  $t = 27 \text{ min}$ ,  $95\% \text{ A}$ ;  $t = 27\text{--}30 \text{ min}$ , column rinse and reequilibration. The flow rate was  $0.7 \text{ ml min}^{-1}$ , and separations were carried out at room temperature. Diode array detection was set at  $280 \text{ nm}$ , and peak identification in breaded deep-fried fish samples was carried out by comparing retention times and spectra of unknown peaks with reference standards.

Increasing levels of HMF standard ( $5.6\text{--}44.2 \mu\text{g}$ ) were added to the coated deep-fried fish samples, which were analysed following the established conditions. Thus, curves (in microgram HMF added per gram sample vs. peak area) were constructed and used for the HMF quantification.

## 2.6. Statistical analysis

Influence of cooking and handling condition (deep-frying in sunflower/olive; oven-baking; reheating in the microwave; time after deep-frying) were determined by analysis of variance (ANOVA). The effect of time and temperature of deep-frying

were analysed according to the General Linear Model. When a significant effect ( $p < 0.05$ ) was detected, paired comparisons between means were conducted using the Tukey's test. Analyses were done by using the SPSS package (v.20.0).

### 3. Results and discussion

#### 3.1. Furanic compound levels as affected by cooking and handling conditions

Table 1 shows the content of furanic compounds in breaded fish products cooked by different methods (deep-frying in sunflower (SF)/olive oil (OL), baking in the electric oven (OV), reheating in microwave after deep-frying in sunflower oil (MO)). The highest levels of total furanic compounds were found in OL products ( $30.36 \mu\text{g g}^{-1}$  sample), followed by SF samples ( $19.46 \mu\text{g g}^{-1}$  sample), whereas OV and MO batches showed the minor content ( $9.99$  and  $8.15 \mu\text{g g}^{-1}$  sample, respectively), due to levels of 2-furfural, furfuryl alcohol, 2-pentylfuran and HMF. However, the highest content of furan was found in SF products ( $5.51 \mu\text{g g}^{-1}$  sample), followed by OV and OL ones ( $4.36$  and  $4.02 \mu\text{g g}^{-1}$  sample, respectively), while this compound was not presented in MO samples. In addition, HMF was also not detected in MO products and 2-furfural was not found in OV samples. In spite of reaching higher heating temperature when oven-baking ( $200^\circ\text{C}$ ) than when deep-frying ( $180^\circ\text{C}$ ), higher levels of furanic compounds were found in SF and OV samples than in OL ones, showing the impact of the oil on the quantity of furanic compounds in deep-fried products. A major evaporation of these volatile compounds occurs in the oven than in the deep-fryer filled with the oil, which could explain this finding. In fact, Van Lancker et al. (2009) reported that oils caused high furan retention. Oil composition also seems to be of importance. Thus, olive oil better enhances the formation of furanic compounds than sunflower oil, which can be related to the higher antioxidant capacity in sunflower than in olive oil before and after frying (Quiles et al., 2002). However, Ramírez et al. (2004) found higher area units of 2-pentylfuran in pork loin samples fried in sunflower than in olive oil. Baking fish breaded products in the electric oven minimized the levels of furanic compounds, although this effect was not so marked in the case of furan. However, Roberts et al. (2008) did not detect furan in packaged convenience foods after cooking in both conventional and microwave ovens. The practise of deep-frying followed by keeping in the fridge and reheating in the microwave reduced the content of furanic compounds, especially of furan and HMF, which could be explained by the evaporation of these compounds during the reheating in the microwave. It also could be due to their losses at low temperatures without being subsequently re-generated in the microwave. This result is in agreement with the studies of furan loss on reheating carried out by Roberts et al. (2008). Nevertheless, HMF

concentration was not significantly different between almonds roasted in the microwave, oven, or oil (Agila and Barringer, 2012). Studies carried out in environmental food contaminants, such as polycyclic aromatic hydrocarbons, polychlorinated dibenzo-p-dioxins and dibenzofurans, have found that the cooking processes are only of limited value as a means of reducing the concentrations of these compounds (Perelló et al., 2009a, 2009b), which mainly depend on the food item (Domingo, 2011).

The influence of time and temperature of deep-frying on furanic compounds levels is exposed in Table 2. As can be observed, total furanic compounds levels significantly ( $p < 0.001$ ) raised as both time and temperature increased, and this trend is also followed by the individual compounds, except for 2-pentylfuran, which was not influenced by the cooking temperature. Thus, the highest values of furanic compounds were found when deep-frying at  $200^\circ\text{C}$  during 6 min ( $38.83 \mu\text{g g}^{-1}$  sample) and the lowest at  $160^\circ\text{C}$  during 2 and 4 min and at  $180^\circ\text{C}$  during 2 min ( $10.65$ ,  $11.76$  and  $11.76 \mu\text{g g}^{-1}$  sample, respectively). These minor quantities are near to those obtained when oven-baking and reheating in the microwave ( $9.99$  and  $8.15 \mu\text{g g}^{-1}$  sample, respectively) (Table 1), and lower in comparison with products deep-fried following manufacturer recommendations ( $180^\circ\text{C}$  during 4 min) ( $19.52 \mu\text{g g}^{-1}$  sample). Thus, it is possible to minimize the generation furanic compounds when deep-frying this kind of products by adjusting the cooking conditions. Agila and Barringer (2012) also found an increase of HMF in almonds when increasing roasting time and temperature. Until now 2-furfural and HMF have been evaluated as indicators of the severity of heat treatment or length of storage in several foods (Gökmen and Acar, 1999; Morales et al., 1997; Teixidó et al., 2006). In addition, results of this study point out that, not only 2-furfural and HMF, also furan and furfuryl alcohol could be used as heat cooking signs in breaded products.

Apart from the influence of the cooking conditions on the generation of furanic compounds, the handling after processing is also of importance. Since these compounds are highly volatile, the decrease in their concentrations by evaporation during domestic handling should be substantial. In this sense, the stability of the furanic compounds after cooking was evaluated in the deep-fried breaded fish products of this study (Fig. 1). The levels of furan significantly decreased with the time (from  $5.51 \mu\text{g g}^{-1}$  in ST0, to  $4.59$  and  $3.68 \mu\text{g g}^{-1}$  sample in ST1 and ST2, respectively). Other authors have also reported that furan is not stable in foods after preparing or opening the commercial products, and that its loss is clearly related to the product temperature, time of exposure to the atmosphere and the food matrix (Goldmann et al., 2005; Kim et al., 2009). The content of furfuryl alcohol kept constant between ST0 and ST1 products ( $10.54$  and  $10.56 \mu\text{g g}^{-1}$  sample, respectively) and decreased in ST2 ones ( $7.16 \mu\text{g g}^{-1}$  sample) (Fig. 1). The levels of 2-furfural and 2-pentylfuran experimented an

**Table 3**  
Abundance (total area count  $\times 10^7$ ) of chemical families of volatile compounds detected in breaded fish products deep-fried in sunflower (SF) and olive (OL) oils, oven-baked (OV) and reheated in the microwave after deep-frying in sunflower oil (MO)\*.

	SF	OL	OV	MO	p
Aldehydes	$22.86 \pm 2.37^a$	$18.28 \pm 0.64^b$	$4.78 \pm 0.56^c$	$7.12 \pm 0.61^c$	<0.001
Aliphatic hydrocarbons	$188.97 \pm 15.69^a$	$7.61 \pm 0.87^b$	$9.63 \pm 0.02^b$	$9.29 \pm 2.09^b$	<0.001
Alcohols	$2.40 \pm 0.19$	ND	ND	ND	<0.001
Ketons	$3.01 \pm 0.76$	$1.96 \pm 0.82$	ND	ND	<0.001
Aromatic hydrocarbons	$8.24 \pm 1.91^b$	$14.50 \pm 1.25^a$	$5.43 \pm 0.55^{bc}$	$3.14 \pm 0.07^c$	<0.001
Furans	$9.36 \pm 1.05^a$	$5.26 \pm 1.33^b$	ND	$2.86 \pm 0.12^c$	<0.001
Pyrazins	$10.96 \pm 1.52^a$	$7.18 \pm 0.79^b$	ND	ND	<0.001
Esters	$2.80 \pm 0.43^a$	ND	$1.54 \pm 0.06^b$	ND	<0.001
Total	$249.36 \pm 22.63^a$	$54.21 \pm 5.74^b$	$22.41 \pm 1.25^c$	$21.98 \pm 2.54^c$	<0.001

Different letters within the same row differed significantly ( $p < 0.05$ ).

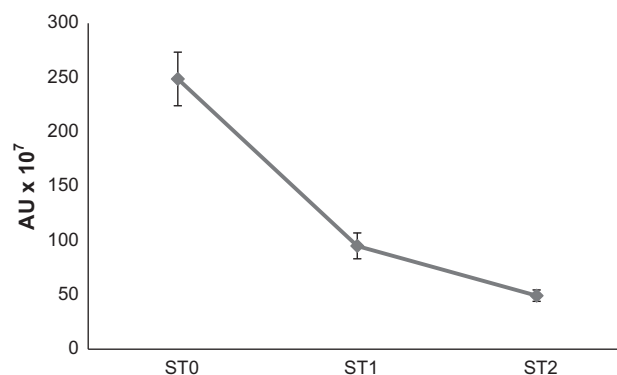
ND: nondetected.

\* The results are expressed as means values  $\pm$  standard deviation.

**Table 4** Abundance (total area count  $\times 10^7$ ) of chemical families of volatile compounds detected in breaded fish products deep-fried at different combinations of time (t) and temperature (T).

	160 °C			180 °C			200 °C			p (t)	p (T)	p (t-T)
	2 min			4 min			6 min					
	2 min	4 min	6 min	2 min	4 min	6 min	2 min	4 min	6 min			
Aldehydes	13.13 ± 0.88 <sup>ef</sup>	11.44 ± 0.45 <sup>f</sup>	35.26 ± 0.85 <sup>c</sup>	15.21 ± 0.10 <sup>c</sup>	22.86 ± 2.37 <sup>d</sup>	69.78 ± 4.16 <sup>a</sup>	18.17 ± 1.49 <sup>de</sup>	23.32 ± 3.37 <sup>d</sup>	61.53 ± 3.15 <sup>b</sup>	<0.001	<0.001	<0.001
Aliphatic hydrocarbons	9.09 ± 0.98 <sup>c</sup>	165.39 ± 70.75 <sup>b</sup>	4.21 ± 0.36 <sup>c</sup>	8.86 ± 1.23 <sup>c</sup>	188.97 ± 15.69 <sup>b</sup>	11.83 ± 0.73 <sup>c</sup>	13.94 ± 0.31 <sup>c</sup>	443.81 ± 118.15 <sup>a</sup>	9.38 ± 1.51 <sup>c</sup>	<0.001	0.029	<0.001
Alcohols	0.93 ± 0.11 <sup>b</sup>	0.87 ± 0.15 <sup>b</sup>	1.27 ± 0.41 <sup>b</sup>	1.49 ± 0.12 <sup>b</sup>	2.40 ± 0.19 <sup>a</sup>	1.43 ± 0.39 <sup>b</sup>	1.23 ± 0.08 <sup>b</sup>	ND	1.11 ± 0.13 <sup>b</sup>	0.736	0.001	<0.001
Ketons	ND	1.71 ± 0.09 <sup>bc</sup>	1.87 ± 0.66 <sup>b</sup>	2.28 ± 0.28 <sup>b</sup>	3.01 ± 0.76 <sup>b</sup>	5.61 ± 0.54 <sup>a</sup>	2.71 ± 0.72 <sup>b</sup>	3.53 ± 1.07 <sup>bc</sup>	5.25 ± 0.83 <sup>ab</sup>	<0.001	<0.001	<0.001
Aromatic hydrocarbons	9.23 ± 0.39 <sup>b</sup>	3.79 ± 0.19 <sup>c</sup>	12.47 ± 1.47 <sup>a</sup>	7.68 ± 0.07 <sup>b</sup>	8.24 ± 1.91 <sup>b</sup>	12.25 ± 0.77 <sup>a</sup>	7.36 ± 0.72 <sup>b</sup>	9.15 ± 1.02 <sup>b</sup>	12.16 ± 0.77 <sup>a</sup>	<0.001	0.401	<0.001
Furans	1.72 ± 0.15 <sup>d</sup>	1.13 ± 0.41 <sup>d</sup>	7.99 ± 0.06 <sup>c</sup>	1.88 ± 0.39 <sup>d</sup>	9.36 ± 1.05 <sup>c</sup>	18.56 ± 1.53 <sup>a</sup>	3.07 ± 0.42 <sup>d</sup>	12.94 ± 1.03 <sup>b</sup>	17.22 ± 0.05 <sup>a</sup>	<0.001	<0.001	<0.001
Pyrazines	0.79 ± 0.26 <sup>d</sup>	ND	5.53 ± 0.82 <sup>c</sup>	ND	10.96 ± 1.52 <sup>b</sup>	20.85 ± 1.66 <sup>a</sup>	0.87 ± 0.06 <sup>d</sup>	12.05 ± 0.25 <sup>b</sup>	20.26 ± 0.13 <sup>a</sup>	<0.001	<0.001	<0.001
Esters	ND	ND	ND	ND	2.80 ± 0.43	ND	ND	2.91 ± 0.27	ND	<0.001	0.014	<0.001
Total	34.84 ± 2.73 <sup>ef</sup>	184.23 ± 71.06 <sup>bc</sup>	68.98 ± 4.87 <sup>d</sup>	36.93 ± 2.08 <sup>ef</sup>	251.79 ± 22.36 <sup>b</sup>	142.36 ± 9.63 <sup>c</sup>	47.21 ± 3.62 <sup>c</sup>	501.14 ± 123.47 <sup>a</sup>	127.6 ± 6.18 <sup>c</sup>	<0.001	<0.001	<0.001

Different letters within the same row differed significantly ( $p < 0.05$ ).  
 ND: **nondetected**.  
 \* The results are expressed as means values  $\pm$  standard deviation.



**Fig. 2.** Abundance of total volatile compounds in deep-fried breaded fish samples analysed immediately after cooking (ST0) and with a delay of 10 (ST1) and 20 min (ST2).

increase between ST0 (0.23 and 1.50, respectively) and ST1 samples (0.68 and 3.38  $\mu\text{g g}^{-1}$  sample, respectively), and decreased in ST2 products (0.22 and 1.15  $\mu\text{g g}^{-1}$  sample, respectively). Thus, the losses of furanic compounds in coated products seem to be more notable 20 min after deep-frying.

### 3.2. Profile of volatile compounds as affected by cooking and handling conditions

A total of 60 volatile compounds were detected, being clustered in the following chemical families: aldehydes (2-methylpropanal; 3-methylbutanal; 2-methylbutanal; hexanal; 2-hexenal; heptanal; benzaldehyde; octanal; benzeneacetaldehyde; 2-octenal; benzaldehyde; nonanal; nonenal; 2-decenal; 2,4-decadienal; 2-dodecenal), alcohols (2-methyl-1-butanol; 1-pentanol; 2-pentanolacetate; 1-hexanol; 1-butanol-3-methyl, acetate; 1-octen-3-ol; 2-ethylhexanol), ketones (2-pentanone; 2,3-pentanedione; 2-heptanone), aliphatic hydrocarbons (2-methylpentane; 3-methylpentane; hexane; heptane; 2-octene; decane; undecane; dodecene; dodecane), aromatic hydrocarbons (benzene; methylbenzene; chlorobenzene; 1,3-dimethylbenzene; ethenylbenzene; 1-ethyl,3,5-dimethylbenzene; limonene; **naphthalene**), esters (acetic acid, ethyl ester; propanoic acid, methyl ester; butanoic acid, methyl ester; butanoic acid, 2-methylproylester; butanoic acid, butyl ester; acetic acid, hexil ester; butanoic acid, 3-methyl, butyl ester; octanoic acid, methyl ester; butanoic acid, hexyl ester; octanoic acid, ethyl ester), furans (furan; 2-furfural; furfuryl alcohol; 2-pentylfuran), pyrazines (methylpyrazine; dimethylpyrazine; ethylpyrazine). Results on the analysis of volatile compounds in this study are expressed as total area counts of the different chemical families.

The effect of different cooking methods on the profile of volatile compounds was also evaluated in breaded fish products (Table 3). The highest levels of total volatile compounds were found in SF products (249 AU  $\times 10^7$ ), followed by OL samples (54 AU  $\times 10^7$ ) and OV and MO batches showing the minor values (22 AU  $\times 10^7$ ), due to most chemical families of volatile compounds showed higher levels in SF than in the other batches. This result shows the notable impact of deep-frying in sunflower oil on the formation of volatile compounds in coated products. In fact, Pokorný (1999) stated that the volatile oxidation products of linoleic acid are the most important flavour compounds found in fried foods, while the oxidation products of oleic acid are less important in contributing to the fried flavour. In addition, these findings could be also explained by the rapid rate that volatile compounds evaporate once they have been formed, being greater in breaded fish products cooked in the oven or reheated in the microwave than in deep-fried products.

The influence of time and temperature of deep-frying on the profile of volatile compounds is exposed in Table 4. As can be seen, the generation of total volatile compounds increased with temperature, and all chemical families presented the same trend. However, the effect of time of deep-frying was not so plain, finding higher levels of volatile compounds when deep-frying during 4 than 6 min, and obtaining the minor levels in coated products deep-fried during 2 min. This result is due to the values of aliphatic hydrocarbons, and specifically to 2- and 3-methylpentane content, showing significant higher values when deep-frying during 4 min at 200, 180 and 160 °C (444, 189 and 165 AU<sub>x</sub> × 10<sup>7</sup>) than when applying the other combinations of time and temperature (from 4.21 to 13.94 AU<sub>x</sub> × 10<sup>7</sup>). Nevertheless, the content of the rest of chemical families of volatile compounds significantly rose as the time increased. This finding indicates a great generation of 2- and 3-methylpentane when deep-frying during 4 min, as well as their degradation when deep-frying during more than 4 min. Due to their high threshold values, the presence of these volatile compounds seems to have a limited influence on products aroma (Ansorena et al., 2001), consequently, differences in these aliphatic hydrocarbons might not influence notably on the flavour of deep-fried breaded fish products.

Apart from the influence of the cooking conditions on the generation of volatile compounds, their stability after cooking was also evaluated (Fig. 2). As can be observed, the total content of volatile compounds significantly decreased from ST0 (252 AU<sub>x</sub> × 10<sup>7</sup>) to ST1 (95 AU<sub>x</sub> × 10<sup>7</sup>), due to the diminishing of aliphatic hydrocarbons, while the rest of chemical families kept their values. From ST1 to ST2 the values of total volatile compounds went on decreasing (95 and 49 AU<sub>x</sub> × 10<sup>7</sup>, respectively) because of most chemical families experimented a significant decrease, however, the levels of polycyclic hydrocarbons maintained throughout this time.

Aldehydes, alcohols, pyrazines, pyridines and furans are thought to play an important role in the flavour of the fried samples (Elmore et al., 1999; Timón et al., 2004). Thus, the effect of cooking and handling conditions on the content of these chemical families of volatile compounds might lead to differences in aroma and flavour attributes of breaded fish products.

#### 4. Conclusions

Cooking and handling conditions exert an important effect on volatile and furanic compounds levels in breaded fish products. Oven-baking and reheating in the microwave are preferred than deep-frying for reducing the generation of furanic compounds. In addition, furanic compound levels might be also minimized by adjusting deep-frying conditions (in sunflower oil at 160 °C – 4 min or 180 °C – 2 min) and the time after cooking (10 min). However, other volatile compounds related to the aroma and flavour of fried food, were also reduced.

#### 5. Uncited reference

Pérez-Palacios et al. (2012c).

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