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Headspace solid-phase Microextraction of Volatile and furanic Compounds in Coated Fish Sticks: Effect of the Extraction Temperature

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Abstract**—**This work evaluated the effect of temperature on headspace solid-phase microextraction of volatile and furanic compounds in coated fish sticks. The major goal was the analysis of the samples as consumed, to reproduce volatile compounds people feel when consuming those products. Extraction at 37 ºC (the human body temperature) throughout the HS-SPME analysis of volatile and furanic compounds in coated fish was compared with higher extraction temperatures, which are frequently used for this kind of determinations. The profile of volatile compounds found in deep-fried (F) and non-fried (NF) coated fish at 37 and 50 ºC was different from that obtained at 80 ºC. Concerning furan and its derivatives, an extra formation of these compounds was observed at higher extraction temperatures. The analysis of volatile and furanic compounds in fish coated sticks simulating the cooking and eating conditions can be reliably carried out setting the headspace absorption temperature at 37 ºC.

Keywords**—**analysis of samples as consumed, fish coated sticks, furans, headspace extraction temperature, volatiles.

I. Introduction

DURING food frying complex processes occur, mainly by lipid oxidation and Maillard reactions, different components react to produce intermediary compounds or volatiles, responsible for the characteristic flavor of fried foods [1]. However, these chemical reactions are also responsible for the formation of undesirable or harmful compounds [1], [2], such us furan and its derivatives.

Furan has been classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer [3], and has been included by the US Department of Health and Human Service [4] in the human pathogen list. However, increased attention is being given to their derivatives, since some of them are considered toxic to animals and humans [5]-[7], thus the quantification of these compounds in cooked foods is of major relevance.

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The solid phase microextraction (SPME) technique has been successfully applied to the analyses of volatile compounds in different types of foods and beverages [8], [9] and it is also appropriate for furan analysis [10], [11]. During the analysis of headspace (HS) by SPME, the effect of some variables, such us type of fiber, sample agitation, sample pH, ionic strength, volume, time and temperature, on the extraction efficiency has been studied in order to determine the optimal conditions [12]-[15]. Several SPME methods use temperatures higher than 50 ºC [12], [13], [16]. However, the use of a high extraction temperature can increase the formation of thermally generated compounds, presenting artifacts in the volatile profile [14].

The first method published for furan analysis indicated extraction at 80 ºC [17], [18]. In 2006, [19] US FDA decreased the extraction temperature for furan analysis from 80ºC to 60 ºC, because it was reported that the furan response increased as the equilibration temperature rose [20]. Since then, different extraction temperatures have been used, namely, HS-SPME at 25 ºC for furan analysis in different Spanish food [21], fiber exposure temperature at 30 ºC for determination of furan in commercial and home-made baby-food [22], and extraction temperature at 50 ºC for furan analysis in canned and jarred food [23]. The use of extraction temperatures close to that of human body temperature is recommended since it reproduces the volatile compounds that people feel when the products are being consumed [12]. EFSA claims that furan analysis must be performed in the samples as they are consumed [11], consequently, it is important to check that the HS-SPME extraction temperature does not influence the furan determination in cooked samples [24]. This work aimed to compare the extraction of volatile compounds with a particular detail on furan and its derivatives from HS at 37 ºC and at higher extraction temperatures in fried fish coated products and simulating the usual preparation and eating process.

II. Materials And Methods

A. Chemicals and standards

Methanol was supplied by Merck (Darmstadt, Germany) and ultrapure water (0.055 µS/cm) was obtained by using a SeralPur Pro 90 CN system. n-Alkanes were purchased by Sigma–Aldrich (St Louis, MO, USA). The deuterated internal standard d_4-furan (98%) was purchased by Isotec (Ohio, USA). Furan (≥99%) and furfuryl alcohol (99%) were supplied by Sigma-Aldrich (Steinheim, Germany) while 2-furfural was provided by Merck (99%) (Darmstadt, Germany) and 2-pentylfuran (98%) was purchased from Alfa Aesar (Karlsrula, Germany).

Frozen coated fish, with 30 g on average, were obtained from a local store, and labeled as being made of fish (65%; *Merluccius capensis*), wheat flour, water, vegetable oil, salt, spices and natural aroma. Plain sunflower oil used for frying was also from a local recognized brand.

B. Sample preparation

Frozen coated fish samples were divided in two groups. The first group (n=18) was thawed at 4 ºC during 16 h and analyzed without being deep-fried (NF). The second group (n=18) was individually deep-fried (F) in sunflower oil using a domestic deep-fryer (Kenwood DF-150; 1l) at 180 ºC during 4 min, according to the manufacturer recommendations. Each sample was fried alone and immediately analyzed for volatiles. The oil was replaced every 5 frying sessions. After each deep-fried, sticks were slightly drained, and placed on paper towel for removing external oil. In order to simulate the mastication process and study the volatiles release through this process, samples were grinded by using a "masticator shears straight" device (Fig. 1) (Bueno Hermanos, S.A., La Rioja, Spain, ISO 9001-2000 Quality Certified Company), usually used for people unable to masticate. Eight bites were patronized.

Six samples of each group were assayed at three different temperatures, 37 ± 1 ºC, 50 ± 1 ºC and 80 ± 1 ºC.

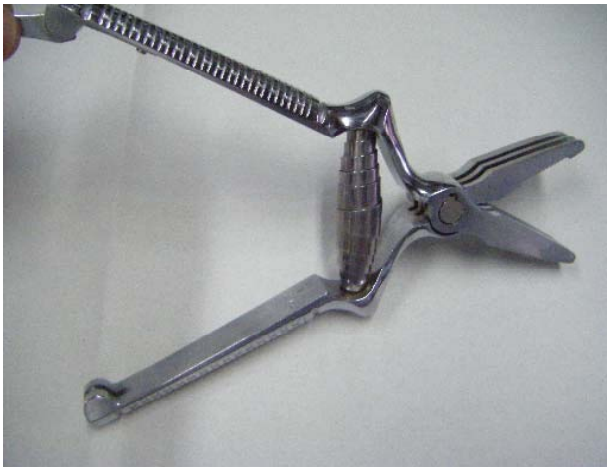

Fig. 1 Manual device used in this study for grinding the coated fish stick products

C. Volatile compounds analysis

The volatile compounds were determined immediately after frying and grinding, by HS-SPME with GC-MS. Briefly, a sample portion (2 g) was transferred to a 50 ml vial containing 5 ml of water and 3 g of NaCl. The vial was sealed at once and kept at – 4 ºC during 10 min, followed by sonication (Fungilab, Portugal) during 15 min at room temperature.

A SPME fiber coated with carboxen–polydimethylsiloxane (CAR-PDMS) (75 μm thickness, Supelco Co., Bellefonte, PA, USA) was used to adsorb the volatile compounds. The SPME fiber was preconditioned at 300 ºC for 60 min, in a gas chromatograph injection port, inserted into the sample vial through the septum and exposed to the HS for 40 min at different temperatures (37 ± 1 ºC, 50 ± 1 ºC and 80 ± 1 ºC) under constant agitation (600 rpm). Thereafter, the SPME fiber was inserted into the injection port and desorbed for 10 min. The injection port was at 280ºC, in the split-less mode, with 1 ml min^{-1} flow Chromatographic analysis was performed using an Agilent 6890 series gas chromatograph (Agilent, Avondale, PA, USA) coupled to a mass selective detector (Agilent 5973). Volatiles were separated using a 5% phenyl-methyl silicone (HP-5) bonded phase fused-silica capillary column (Hewlett-Packard, Palo Alto, CA, USA; 33 m x 250 μm i.d., film thickness 0.25 μm), operating with helium at 80 kPa column head-pressure, resulting in a flow of 1 ml min^{-1} at 40 ºC. The oven temperature program was isothermal for 5 min at 40 ºC, raised to 135 ºC at a rate of 3 ºC min^{-1} and then raised to 220 ºC at 20 ºC min^{-1}. The transfer line to the mass spectrometer was maintained at 250 ºC. Mass spectra were obtained by electronic impact at 70 eV, with a multiplier voltage of 2056 V, collecting data in the fullscan mode at a rate of 1 scan s^{-1} over the *m/z* range 30–500. n-Alkanes (Sigma, St Louis, MO, USA) were run under the same chromatographic conditions to calculate the retention indices (RI). Volatile compounds were identified by comparison of their mass spectrum with reference compounds in the NIST 98 data bank (NIST/EPA/NISH Mass Spectral Library, version 1.6, U.S.A.), and by comparison of RI with those described in the literature [8], [16], [25], [26]. Individual volatile compounds were determined in area units, and results of the present study were based on the relative percentages of the chemical families of volatile compounds detected. The furanic compounds were also quantified as described below.

D. Furanic compounds quantification

External calibration curves were used for quantification of furanic compounds. A standard calibration solution containing furan, 2-furfural, furfuryl alcohol and 2-pentylfuran at 8.69, 0.52, 10.84 and 0.07 mg ml^{-1}, respectively, and a d_4-furan solution at 1 μg μl^{-1} were prepared. Five consecutive dilutions of the standard calibration solution in methanol (1:10 v/v) were made. Portions of 100 μl of each standard solution and a fixed volume (100 μl) of d_4-furan solution were prepared as the samples, extracted by the SPME fiber and injected into the gas-chromatograph. The *m/z* used for the quantification of the furanic compounds were *m/z* 68, *m/z* 72, *m/z* 96, *m/z* 98 and m/z 138 ions for furan, d_4-furan, 2-furfural, furfuryl alcohol, and 2-pentylfuran, respectively. 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 ug g^{-1}, take into account the exact weight of the sample portion in the vial.

E. Statistic analyses

The effect of HS extraction temperature on volatile and furanic compounds of fish coated sticks was analyzed by one-way analysis of variance (ANOVA). Analyses were done by using the SPSS package (v.15.0).

III. Results And Discussion

A. Profile of volatile compounds in fish coated sticks as a function of the HS extraction temperature

A total of 60 volatile compounds were detected in NF and/or F samples (Table I), being clustered in the following chemical families: aldehydes, alcohols, ketones, aliphatic and aromatic hydrocarbons, esters, furans and pyrazines. Fig. 2 shows the percentage of aldehydes, ketons, furans and pyrazines extracted at 37, 50 and 80 ºC by the SPME fiber, in NF and F coated fish sticks, and Figure 3 presents the percentage of esters, alcohols, aromatic and aliphatic hydrocarbons extracted at 37, 50 and 80 ºC by the SPME fiber, in NF and F coated fish sticks. Figures 2 and 3 highlight that aldehydes, ketons, furans and pyrazines increased significantly ($p < 0.05$) from NF to F products whereas esters and aromatic hydrocarbons decreased ($p < 0.05$) from NF to F products. Aliphatic hydrocarbons and alcohols did not show significant differences between NF and F samples. The changes in volatile composition can be related with i) volatiles taken from the cooking oil; ii) the compounds thermally generated or degraded in the coated fish and oil during frying; iii) the compounds formed as a results of interaction between food and oil compounds at high temperature, namely Maillard products [16].

The effect of the HS extraction temperature on the percentage of each chemical family of volatile compounds was significant, being similar in both NF and F samples (Figures 2 and 3). Values of aldehydes, alcohols, furans, esters, pyrazines and aromatic hydrocarbons showed a significant increase as the HS temperature rose, whereas the content of aliphatic hydrocarbons decreased with the increase of temperature. The percentage of ketons increased from 37 to 50 ºC and decreased (in F samples) or maintained (in NF samples) from 50 to 80 ºC. As consequence, different profile of volatile compounds in the coated fish sticks was found depending on the HS extraction temperature, i.e. in the F group extracted at 80 ºC, aldehydes were the major chemical family, followed by aliphatic hydrocarbons and the rest of chemical groups showing minor percentages, whereas at 37 and 50 ºC of HS temperature, the major chemical family was aliphatic hydrocarbons, followed by aldehydes.

TABLE I
Volatile Compounds Detected In Nf and/or F Samples Grouped by Chemical Families

ALDEHYDES	ALCOHOLS
2-methylpropanal	2-methyl-1-butanol
3-methylbutanal	1-pentanol
2-methylbutanal	2-pentanolacetate
hexanal	1-hexanol
2-hexenal	1-butanol-3-methyl, acetate
Heptanal	1-octen-3-ol
2-heptenal	2-ethylhexanol
benzaldehyde	ALIPHATIC HYDROCARBONS
octanal	2-methylpentane
benzeneacetaldehyde	3-methylpentane
2-octenal	hexane
nonanal	heptane
nonenal	2-octene
2-decenal	decane
2,4-decadienal	undecane
2-dodecenal	dodecene
	dodecane

KETONS	ESTERS
2-pentanonone	acetic acid, ethyl ester
2,3-pentanedione	propanoic acid, methyl ester
2-heptanone	butanoic acid, methyl ester
	butanoic acid, 2-methylprotylester
FURANS	butanoic acid, butyl ester
furan	acetic acid, hexil ester
furfural	butanoic acid, 3-methyl, butyl ester
furfurylalcohol	octanoic acid, methyl ester
2-pentylfuran	butanoic acid, hexyl ester
	octanoic acid, ethyl ester

PYRAZINES	AROMATIC HYDROCARBONS
methylpyrazine	benzene
dimethylpyrazine	methylbenzene
ethylpyrazine	chlorobenzene
	1,3-dimethylbenzene
	ethenylbenzene
	1-ethyl,3,5-dimethylbenzene
	limonene
	naphthalene

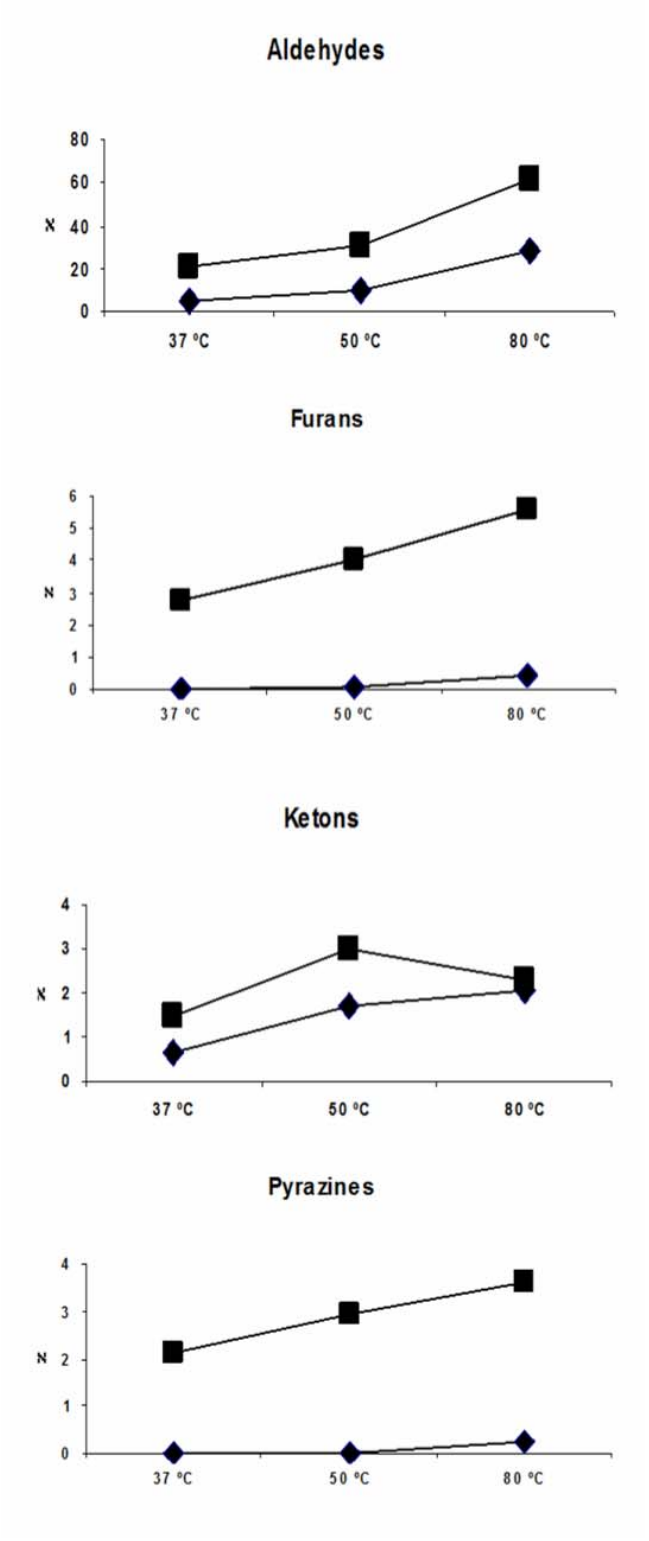

Fig. 2 Effect of headspace extraction temperature (37 ºC; 50 ºC; 80 ºC) on the percentage of aldehydes, furans, ketons, and pyrazines from ◆ non-fried (NF) and ■ deep-fried (F) coated fish samples

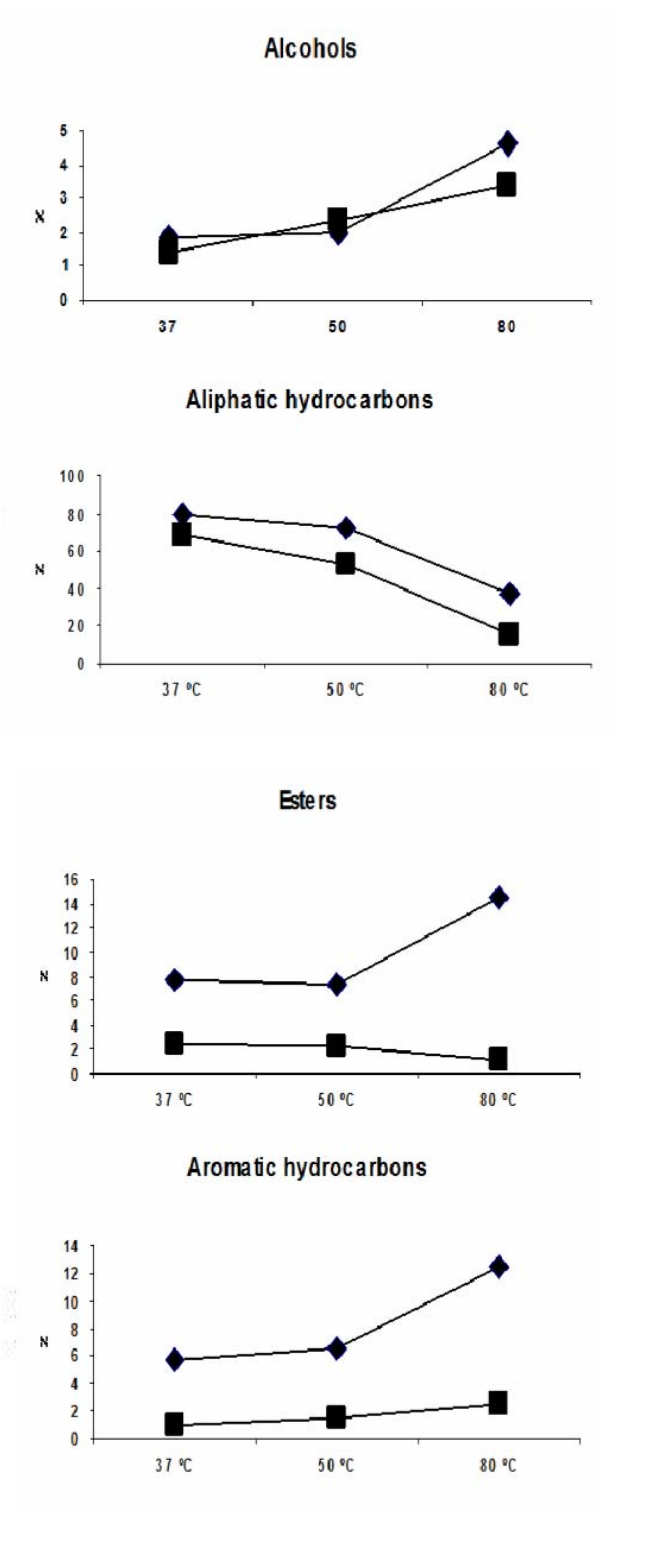

Fig. 3 Effect of headspace extraction temperature (37 ºC; 50 ºC; 80 ºC) on the percentage of alcohols, aliphatic hydrocarbons, esters, and aromatic hydrocarbons from ◆ non-fried (NF) and ■ deep-fried (F) coated fish samples

B. Quantification of furanic compounds in coated fish sticks as a function of the HS extraction temperature

Table II shows the quantity of furan and its derivatives (furan, 2-furfural, furfuryl alcohol and 2-pentylfuran) extracted from NF and F products at 37, 50 and 80 ºC. Furan, 2-furfural, and furfuryl alcohol were not detected in NF samples sampled at 37 and 50 ºC. Only 2-pentylfuran was found in NF samples at these lower temperatures. At 80 ºC 2-furfural, furfuryl alcohol and 2-pentylfuran were quantified in NF. The quantity of 2-furfural, furfuryl alcohol and 2-pentylfuran in F samples increased as the extraction temperature rose, whereas the levels of furan increased from 37 to 50 ºC, but it was not detected when the HS extraction temperature was set at 80 ºC. These results point out i) the extra formation of furanic compounds with the increase of HS temperature, and ii) the furan decomposition and/or difficulty of extraction at high HS temperatures. Extraction temperatures between 37 and 50 ºC reduce these effects. Comparison with results reported in literature is difficult, because contradictory results about influence of temperature on furan extraction are described. In canned food, an increase in the HS extraction temperature from 30 to 50 ºC caused an increase in the furan peak area [5], and furan peak area increased exponentially as the equilibration temperature increased from 40 to 80 ºC in green coffee, tomato juice and orange juice [20]. But other authors found a decrease in the furan response in canned and jarred food samples when increasing the extraction temperature from 25 to 40 ºC [21]. Probably, the effect of the extraction temperature on furan chromatographic peak area depends on the kind of product analyzed. With respect to 2-furfural, furfuryl alcohol and 2-pentylfuran, no studies were found in the literature concerning the influence of the extraction temperature on the content of these furanic compounds.

IV. Conclusions

Headspace extraction temperature influenced the profile of volatile compounds as well as the quantity of furan and its derivatives in coated fish products analyzed before and after deep-frying. Most volatile compounds increased their response as the temperature rise. Extraction temperatures between 37-50 ºC are recommended, extra furanic compounds are formed when extraction is performed at 80 ºC. The analysis of volatiles including furan and its derivatives in coated fish sticks products simulating the cooking and eating conditions can be reliably carried out setting the headspace extraction temperature at 37 ºC. Furfuryl alcohol was the most abundant furan derivative in fried coated fish products, hazards' associated with its presence in fried foods should be explored.

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