Changes in chemical composition of frozen coated fish products during deep-frying

Journal:	*International Journal Of Food Sciences & Nutrition*
Manuscript ID:	Draft
Manuscript Type:	Research Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Pérez-Palacios, Trinidad; University of Extremadura, ; Universidade do Porto, Petisca, Catarina; Universidade do Porto, Casal, Susana; Universidade do Porto, Ferreira, Isabel; Universidade do Porto,
Keywords:	Amino acids, Fatty acids, Volatile compounds, Furanic compounds, Deep-frying, Frozen coated products

TITLE

Changes in chemical composition of frozen coated fish products during deep-frying

AUTHORS

Trinidad Pérez-Palacios*[,a,b], Catarina Petisca, Susana Casal[a], Isabel M.P.L.V.O. Ferreira[a]

[a] REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha 164, 4099-030 Porto, Portugal

[b] Tecnología de los Alimentos. Facultad de Veterinaria. Universidad de Extremadura. Avda. de la Universidad s/n, 10071, Cáceres, Spain (permanent address)

* Corresponding author. Tel.: +34 927 257123; fax: +34 927 257110. E-mail address: triny@unex.es (T. Pérez-Palacios).

ABSTRACT

This work evaluates the influence of deep-frying coated fish products on total fat, fatty acid (FA) and amino acid profile, and on the formation of volatile compounds, with special attention on furan and its derivatives due to their potential harmful characteristics. As expected, deep-frying in sunflower oil increased linoleic acid content, but total fat amount increased only by 2% on a dry basis. Eicosapentanoic and docosahexanoic acids were preserved while γ- and α-linoleic acids were oxidized. Deep-frying also induces proteolysis, releasing free AA, and the formation of volatile compounds, particularly aldehydes and ketones arising from polyunsaturated FA. In addition, during deep-frying coated fish high quantities of furanic compounds are generated, particularly furan and furfuryl alcohol. The breaded crust formed could contribute simultaneously for the low uptake of fat, preservation of long chain n-3 FA, and for the high amounts of furanic compounds formed during the deep-frying process.

KEY WORDS

Fatty acids; amino acids; volatile compounds; furanic compounds; deep-frying; coated fish products.

INTRODUCTION

Coated deep-fried fish sticks are highly appreciated, particularly among the younger's, due to their sensorial attributes, quick and easy preparation, and association with good health characteristics (i.e. high n-3 fatty acid (FA) content). As with other fried foods, however, its unique sensorial attractiveness is obscured by the recurring alerts to decrease the consumption of fried foods, mainly due to their large amount of calories provided from fat (Saguy and Dana, 2003).

Deep-frying is a cooking method that induces significant modification in food, including loss of constitutional water, fat uptake, changes in the FA profile (Ramírez, et al., 2004) and the development of heat-induced chemical reactions (Bastida and Sanchez-Muniz, 2001; Romero, Cuesta and Sanchez-Muniz, 2000).

The development of aroma and flavour in fried foods is a complex process in which different compounds react to produce intermediary compounds or volatiles, which mainly derive from lipid oxidation and Maillard reactions (Mottram, 1998). Thus, FA and amino acids (AA) are probably among the most important precursors of volatiles in this processing (Ramírez, et al., 2004; Huey, Abdul and Mohamed, 2008). Nevertheless these reactions are simultaneously responsible for the formation of undesirable or harmful compounds (Mottram, 1998; Nawar, 1998). A large number of papers related to volatile compound profiles of fried food, mainly meat, have been published (Ramírez, et al., 2004; Timón, et al., 2004; Ho, Lee and Jin, 1983), while the simultaneous

evaluation of FA, AA and potentially harmful volatile compounds, such as furan and its derivatives, remain less studied.

Furanic compounds are recognized as important for the odour characteristics 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 FA (PUFA). However, according to *in vivo* studies, some volatile furan derivatives are considered toxic to animals and humans (Sujatha, 2008; Arts, et al., 2004; IARC, 1995). Up to date, only furan has been classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC 1995), and has been included by the US Department of Health and Human Service in the human pathogen list (US FDA, 2005). An increased attention is being also devoted to their derivatives, being of great importance the quantification of these compounds in food. In accordance, the European Food Safety Authority claims for testing of furan quantities in foods, comprising analysis of samples as consumed, with an exact detail on cooking preparation time, temperature and handling information (EFSA, 2010).

The main goal of the present work was to study the effect of deep-frying of coated fish products on the FA and AA composition and formation of volatile compounds, with a particular detail on furanic compounds.

MATERIAL AND METHODS

Chemicals and standards

Chloroform, methanol, n-heptane, dichloromethane, NaCl and KOH were supplied by Merck (Darmstadt, Germany). Hydrochloric acid (HCl), 32% extra pure, and MTBSTFA (N-Methyl-N-tert-butyldimethylsilyltrifluoroacetamide) were purchased by Sigma–Aldrich (St Louis, MO, USA). Acetonitrile of high performance liquid chromatography (HPLC)-gradient grade was provided by Panreac (Barcelona, Spain) 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) and FA methyl ester (FAME) standards were from Supelco (Bellefonte, 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 (99%) was provided by Merck (Darmstadt, Germany) and 2-pentylfuran (98%) was purchased from Alfa Aesar (Karlsrula, Germany). Standard amino acids (Sigma-Aldrich, Madrid, Spain) purchased for preparing the standard solutions were alanine, glycine, valine, leucine, isoleucine, proline, methionine, serine, threonine, phenylalanine, aspartic acid, hydroxyproline, cysteine, glutamic acid, arginine, asparagine, lysine, glutamine, histidine, tyrosine, tryptophan, and cystine. DL-Norleucine (Sigma-Aldrich) was used as internal standard. Frozen coated fish, with 30 g on average, were obtained from a local store, and labelled 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.

Experimental design

Frozen coated fish samples were divided in two groups. The first group (n=10) was used as control, being thawed at 4 ºC during 16h and analysed without being deep-fried (NF). The second group (n=10) 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 analysed for volatiles. The oil was replaced every 5 frying sessions. After each deep-fried, sample was slightly drained, and placed on paper towel for removing external oil. Samples were grinded by using a "masticator shears straight" device (BUENO HERMANOS, S.A., La Rioja, Spain, ISO 9001-2000 Quality Certified Company), usually used for people unable to masticate.

Moisture determination

Moisture was determined at 100 ± 2 ºC (~5 g test sample) by AOAC 925.40 method (AOAC, 1995).

Lipid extraction

Lipids were extracted following the Folch, Less and Sloane (1957) method, with slight modifications, using the original extraction ratio of 20 parts chloroform:methanol (2:1, v/v) to 1 part sample. Briefly, 0.5 g of sample were mixed with 10 ml of the organic solvents mixture with BHT

0.01%. The mixture was vigorously homogenized and filtered. Subsequently, 2 ml of 1% aqueous NaCl solution were added, the mixture was shaken again and the final byphasic system was allowed to separate by centrifugation (10 min, 1549 xg; 5810R Centrifuge, Eppendorf AG, Hamburg, Germany). The upper aqueous phase was discarded and the lower organic phase was filtered through anhydrous sodium sulphate. Lipid content was estimated gravimetrically after solvent evaporation under nitrogen and reserved for subsequent FA profile.

Fatty acid methyl esters preparation and analysis

Fatty acid methyl esters (FAME) from acyl chains were prepared by basic transesterification of the extracted lipids in the presence of KOH (KOH in methanol 2M) (ISO 5508:2000). FAME were analyzed by gas chromatography, using a Chrompack CP9001 (Middelburg, the Netherlands) gas chromatograph, equipped with a flame ionization detector (FID). Separation was carried out on a capillary column CP-Sil 88 (50 m long, 0.25 mm id, and 0.2 μm film thickness, Varian). Oven temperature programming started at 140° C. After three min, it was raised 5 °C min^{-1} to 220 °C, and held for 8 min at 220 °C. Injector and detector temperatures were 270 and 250 °C, respectively. The carrier gas was helium at 110 KPa. Individual FAME peaks were identified by comparing their retention times with those of standards (Sigma, St. Louis, MO). Determination was based on the relative percentages of the FAME analysed.

Amino acid analysis

Sample (1 g) was weighed into an 15 ml flat-bottom glass vial, and 7.5 mL of 0.1 N HCl were added. After a stir bar had been inserted, the vial was sealed with a screw-top cap and stirred at high speed for 90 min at 40 °C. The mixture was then centrifuged (5000 rpm, 30 min), and the supernatant was transferred into another 15 ml graduated vial, flushed until 10 ml with distilled water and centrifuged again (10000 rpm, 15 min). 1 ml of the supernatant were collected and stored at - 80 °C until analysis.

To 100 µl of the extract, 250 µl of acetonitrile were added to deproteinize the samples. Tubes were subsequently centrifuged at 8000 rpm for 5 min. From this point, standard solutions and food samples followed the same process. Then, 100 µl of the supernatant (or the standard solution) were transferred to heat-resistant tubes, and 100 µl of a DL-norleucine solution (5 µg ml^{-1}) was added as internal standard. Tubes were dried under nitrogen. 50 µL of dichloromethane were added to the dried samples, and again evaporated under nitrogen. Finally, 50 µl of the derivatization agent (MTBSTFA) and 50 µL of acetonitrile were added to the dried tubes, which were shaken and subsequently incubated at 100 °C for 60 min to induce the derivatization reaction to occur. The chromatographic analysis was carried out in Agilent 6890 gas chromatograph (GC) (Agilent, Avondale, PA, USA) coupled to a mass selective (MS) detector (Agilent 5973). 1 µL portion of the derivatized extract was injected in splitless mode onto the column. The column used

was a 58 m × 0.32 mm i.d., 0.05 µm, HP-5 (Hewlett-Packard), being a 5% phenyl-methyl polysiloxane bonded phase fused silica capillary column. Column head pressure was 12.8 psi, resulting in a flow of 1.2 mL/min at 280 °C. The oven program was as follows: 170 °C for 5 min, 4 °C/min ramp to 200 °C, held at 200 °C for 3 min, 4 °C/min ramp to 290 °C, held at 290 °C for 1 min held for 16 min. The transfer line to the mass spectrometer program was as follows: 280 °C for 35 min, 0.5 °C/min ramp to 290 °C. Total run time was 55.75 min. Free AA were identified using both their retention time and by comparison of their characteristic *m/z* ions with those published in the literature (Jiménez-Martín, et al., 2012).

Free AA quantification in coated samples was carried out in the total ion chromatogram (TIC) mode by external calibration curve method. For each AA a calibration curve (AA peak area/DL-norleucine peak area versus AA amount/DL-norleucine amount) was constructed, obtaining R^2 values of 0.9999. The final results, expressed in mg per g dry matter, take into account the moisture content and the exact weight of the sample.

Volatile compound analysis

The volatile compounds were determined immediately after frying and grinding, by head-space solid-phase micro extraction (SPME) with gas-chromatography-mass spectrometry (GC-MS) (Pérez-Palacios, et al., 2012). 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 °C during 10 min, followed by sonication (Fungilab, Portugal) during 15 min at room temperature.

A SPME fibre coated with carboxen–polydimethylsiloxane (CAR-PDMS) (75 µm thickness, Supelco Co., Bellefonte, PA, USA) was used to adsorb the volatile compounds. The SPME fibre was preconditioned at 300 °C for 60 min, in a gas chromatograph injection port, inserted into the sample vial through the septum and exposed to the head-space for 40 min at 37 ± 1 °C under constant agitation (600 rpm). Thereafter, the SPME fibre was inserted into the injection port and desorbed for 10 min. The injection port was at 280°C, 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 °C. The oven temperature programme was isothermal for 5 min at 40 °C, raised to 135 °C at a rate of 3 °C min^{-1} and then raised to 220 °C at 20 °C min^{-1}. The transfer line to the mass spectrometer was maintained at 250 °C. 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 (Ramírez, et al., 2004; Timón, et al., 2004; Muriel, et al., 2004; Pérez-Palacios, et al., 2010). Results of the volatile analysis are given in area units (AU) of each individual compound, except for the furanic compounds that were quantified as described below.

Furanic compounds quantification

Furanic compounds were quantified by means of external calibration curves (Pérez-Palacios et al., 2012). For that, a standard calibration solution of furan, 2-furfural, furfuryl alcohol and 2-pentylfuran at *ca*. 8.69, 0.52, 10.84 and 0.07 mg ml^{-1}, respectively, and a d_4-furan solution at *ca*. 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 samples, adsorbed in the SPME fibre as 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 head-space vial.

Statistical analysis

The effect of deep-frying on moisture, lipid content, FA and AA profile, formation of volatile compounds, and quantity of furanic compounds of coated fish was analysed by one-way analysis of variance (ANOVA). Pearson Correlation between moisture and lipid content was also carried out. Analyses were done by using the SPSS package (v.15.0).

RESULTS AND DISCUSSION

Moisture and lipid content in coated fish products

As expected, F coated products had significant lower ($p<0.018$) moisture levels (52.95 ± 1.33 g water per 100 g fresh food) and higher lipid content (10.48 ± 1.22 g per 100 g dry matter of edible food) than the NF group, with 59.84 ± 0.84 g water per 100 g fresh food and 8.54 ± 1.37 g per 100 g dry matter of edible food, respectively. After immersing samples into the hot oil, surface temperature of coated fish rapidly rises, evaporating the surface water, and leaving voids for the oil to fill in (Mellema, 2003). A considerable number of factors have been implicated in fat uptake, i.e. moisture content, microstructure of the crust formed in the product, temperature and time of processing and the conditions after removal (Mellema, 2003; Mehta and Swinburn, 2001; Ufheil and Escher, 1996). Some authors argue that the total volume of fat

will be similar to the total water volume removed (Pinthus, Weinberg and Saguy, 1993), whereas other have observed that fat uptake are inversely related to moisture content during deep frying (Moreira, Palau and Sun, 1995; Southern et al., 2000). In our study, significant and negative Pearson coefficient (- 0.98) was obtained between lipid and moisture contents, with higher moisture associated with lower fat amounts. The crusted barrier created within the deep-frying processing could reduce the internal moisture loss and confine the fat absorption to the crust, justifying the low fat increase, only around 2%. In addition, the common practice of shaking and draining the oil after frying (as carried out in the present study) is known to have a large effect on total fat uptake (Mellema, 2003).

Effect of deep-frying on FA composition of coated fish

Table 1 shows the FA profile (expressed as g per 100 g FAME) in the NF and F coated fish products analysed. A clear dominance of the FA from the vegetable oils used in the coating (NF), together with those from the sunflower oil (F), is observed. In the NF group, linoleic (C18:2 n-6, 42.51 g per 100 g FAME) and oleic (C18:1 n-9, 40.75 g per 100 g FAME) acids were the major FA found, followed by palmitic (C16:0, 8.35 g per 100 g FAME) and stearic (C18:0, 3.81 g per 100 g FAME) acids. After frying, a clear increase in linoleic acid (C18:2 n-6, 52.4 g per 100 g FAME) is observed, as a direct impact from the FA composition of sunflower oil, with a consequent decrease in the relative proportion of most FA, some with statistical significance (Table 1). Eicosapentaenoid acid (EPA, C20:5

n-3) and docosahexaenoic acid (DHA, C22:6 n-3) were highly stable during the frying process (Table 1), indicating their protection through the process. The bread crust formed during frying might be implicated in this protective effect. On the other hand, γ- and α-linoleic acids (C18:3 n-6 and C18:3 n-3, respectively), being essential FA, were clearly reduced during frying, being probably thermally oxidized during frying, as usually expected with PUFA. These results are in agreement with other authors (Ramírez, et al., 2004; Sánchez-Muniz, Viejo and Medina, 1992). The significantly higher (p<0.001) PUFA content observed after frying (45.95 vs 54.36 g per 100 g FAME) should be interpreted with caution as it is mostly due to an increase in n-6 FA from the frying oil (Table 1). Also, the increased n-6/n-3 ratio reflects the sunflower oil uptake and α-linolenic acid (C18:3 n-3) loss, not directly correlated with fish lipids. The long-chain n-3 FA, as mentioned, were preserved.

Effect of deep-frying on the content of free AA of coated fish

The content of free AA in NF and F samples is exposed in Table 2. Nineteen AA were detected: alanine, glycine, valine, leucine, isoleucine, proline, methionine, threonine, phenylalanine, aspartic acid, cysteine, glutamic acid, arginine, asparagine, lysine, glutamine, histidine, tyrosine and tryptophan. Cysteine was the major AA (19.15 and 46.01 mg per g of edible food in NF and F samples, respectively), followed by phenylalanine (2.96 and 13.04 mg per g of edible food in NF and F samples, respectively) and aspartic acid (3.22 and 6.14 mg per g of edible food in NF and F samples, respectively), while the others showed

minor content (from 0.08 to 0.55 mg per g of edible food and from 0.13 to 0.78 per g of edible food in NF and F products, respectively). However, cysteine has not been found in different fish species (Ozden and Erkan, 2011). Sum of total AA showed a significant increase from NF (30.20 mg per g of edible food) to F coated products (72.47 mg per g of edible food), due to the content of most AA rose during deep-frying, and above all the major ones, which indicates the occurrence of proteolysis during the deep-frying process. As our knowledge, in literature there are no studies measuring free AA content in deep-fried food.

Effect of deep-frying on volatile compounds of coated fish

Table 3 shows data obtained from the analysis of volatile compounds of NF and F coated fish products studied, all quantified on the basis of their direct area counts. A total of 65 volatile compounds were detected, being clustered in the following chemical families: aldehydes, alcohols, ketones, aliphatic and aromatic hydrocarbons, esters, furans and pyrazines. Figure 1 shows the percentage of each chemical family in the two batches of coated fish products of the present study. Aliphatic hydrocarbons were the main family in both NF (79.04 %) and F (71.48 %) groups, being 2-methylpentane and hexane the major individual volatile compounds. However, 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). The second major chemical families in NF products were esters, aromatic hydrocarbons and aldehydes (8.05, 5.81 and 4.56 %, respectively),

followed by alcohols (1.84 %) and ketones (0.68 %). In F samples aldehydes were the second major chemical family (17.69 %), being all the other chemical families between 1-2 %. As can be seen, furan and pyrazine chemical families were only found in F coated fish products.

The detection of volatile compounds in NF products could be explained by i) their formation during the thermally treatment applied to this kind of products before packing; ii) their direct accumulation in muscle fat from feeding, such as hydrocarbons (Sahidi, Rubin and D'Souza, 1986); iii) the proteolytic, lipolytic and oxidative reactions which could take place during the frozen-thawed storage, i.e. 2-ethylhexanol, which can be originated from lipid oxidation or amino acid degradation (Stanke, et al., 2002).

As can be observed in Table 3, significant differences were found in the profile of volatile compounds between NF and F samples, which could be related to i) volatiles 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 (García, et al., 1991).

The content of total aldehydes were significantly higher (p=0.040) in F (101.99 x 10^7 UA) than in the NF group (29.80 x 10^7 UA). The same result was obtained for ketones (p = 0.039), with higher values in F (7.97 x 10^7 UA) compared to NF group (4.46 x 10^7 UA). This was probably due to the heat-induced oxidation of FA from frying oil and food. Aliphatic

aldehydes derived from oxidative degradation of PUFA, have low odour threshold values and may play an important role in the flavour of the fried samples (Elmore, et al., 1999). Nawar (1998) found that the largest amount of volatile compounds produced in oil during frying reflects the major FA of this product, which could explain the significant higher content of 2,4-decadienal in the F samples. This aldehyde originates from the oxidation of linoleic acid (C18:2 n-6), the major FA present in both sunflower oil and coated fish samples. Ketones arise also from oxidation of unsaturated lipids (Sahidi, Rubin and D'Souza, 1986) and some of them give interesting aromatic notes, such 2-heptanone (Ruiz, Muriel and Ventanas, 2002).

Esters are formed by esterification of carboxylic acids and alcohols (Sahidi, Rubin and D'Souza, 1986). This chemical family showed significant higher levels (p=0.002) in NF (51.83 UA x 10^7) than in F group (13.85 UA x 10^7), due to the detection of only three individual esters in F samples against seven in NF samples, indicating the loss of these volatile compounds during deep-frying. In fried loin chops, Ramírez, et al., (2004) did not found esters. In fact, esters have not been described as responsible for the aroma of fried samples (Timón, et al., 2004).

The content of total aromatic hydrocarbons was also significant higher (p<0.002) in NF (37.60 UA x 10^7) than in F group (7.60 UA x 10^7). The reduction was mainly due to limonene loss, whose presence could be associated with the use of aromas and vegetal oils as ingredients in this

coated-fish formulation. During frying this compound may evaporate, decompose or interact with other components.

Regarding total aliphatic hydrocarbons and alcohols, not significant differences between NF and F samples were observed. Some individual volatile compounds were only detected in NF or F batches, reducing their statistical significance. Straight chain alcohols come from PUFA and have an important contribution to the flavour of fried samples (Timón, et al., 2004). Aliphatic hydrocarbons with less than 10 carbon atoms arise mainly from lipid oxidation (Ruiz, Muriel and Ventanas, 2002), while those with longer chains are found accumulated in the fat depots of the muscle animal, resulting probably from feeding (Meynier, et al., 1999). Thus, it can indicate a potential balance during the deep-frying process between the formation of some aliphatic hydrocarbons and alcohols and the loss of others through evaporation or decomposition, as well as the stability of other of these volatile compounds, especially 2 and 3-methylpentanal.

Furans and pyrazines were only detected in F samples, which can be explained by the formation route of these volatiles compounds, associated with thermal treatment during food cooking (Mottram, 1998). Pyrazines are products from Maillard reactions and show very interesting flavours, such as 2,5-dimethylpyrazine, associated to roasted and bready aroma (Timón, et al., 2004). Furans are mainly associated with a sweet, nutty and caramel-like odour impression (Ho, Lee and Jin, 1983). In agreement with our results, Ramírez, et al., (2004) detected 2-

pentylfuran, 2,5-dimetylpyrazine and 2-ethyl-3,5-dimetylpyrazine in fried pork loin but not in raw samples.

Quantification of furanic compounds

Aware of the importance of these compounds and the need for accurate quantification of their amounts in food, 4 furanic compounds were quantified as described in the experimental session: furan, 2-furfural, furfuryl alcohol and 2-pentylfuran. As detailed previously, no furans were detected in the NF samples. The furanic compounds were formed during frying, with higher amounts of furfuryl alcohol (22.28 ± 2.14 µg per g dry matter of edible food) and furan (11.88 ± 2.62 µg per g dry matter of edible food), followed by 2-pentylfuran (3.76 ± 0.20 µg per g dry matter of edible food) and 2-furfural (0.57 ± 0.12 µg per g dry matter of edible food), with a global estimated amount above 34 µg per g dry matter of edible food.

In comparison with other foods described in the literature, the 2-furfural content in the samples of this work was similar to that reported in baby foods (Mesías-García, Guerra-Hernández and García-Villanova, 2010). The quantities of 2-pentylfuran were also comparable to that found in crispbread and mock-turtle (EFSA, 2009). Lower levels of furfuryl alcohol were obtained in the present study than in coffee samples (Swasty and Murkovic, 2011). The furan levels in coated deep-fried fish were higher than those usually reported in coffee beverage, baby food, and soups, some of the foods showing the highest furan content (EFSA, 2010). Becalski, et al., (2005) reported that furan can be formed through

thermal treatment from Maillard precursors or lipids and from pyrolysis of carbohydrates. All these potential precursors occur in fried coated fish, explaining the high content of furan found in this product. These results point out the notable influence of the deep-frying process on the formation of these potentially harmful furanic compounds.

CONCLUSIONS

Deep-frying coated fish products does not exert a large impact on fat intake but increases total PUFA content, specially linoleic acid (C18:2 n-6), and preserves long chain n-3 PUFA from oxidation. However, γ- and α-linoleic acids (C18:3 n-6 and C18:3 n-3, respectively) are oxidized during this culinary process. Proteolysis is also observed during the deep-frying of coated fish products. It also releases several characteristic volatile compounds and leads to high quantities of furanic compounds, especially furan and furfuryl alcohol. The crusted barrier formed during frying could reduce oil uptake and preserve oxidation of long chain n-3 PUFA. On the other hand, it might be also the responsible for the formation of furanic compounds, in amounts justifying attention. Thus, further studies on possible strategies for reducing the formation of potentially harmful compound in this kind of products while keeping or even increasing desirable nutritional and sensorial characteristics are necessary.

Declarations of Interest

Authors declare not to have any conflict of interest.

This work has been supported by Fundação para a Ciência e a Tecnologia through Project PTDC/AGR-ALI/101583/2008 and PEst-C/EQB/LA0006/2011, by funds provided by the Junta de Extremadura-Consejeria de Economía, Comercio e Innovación, and the Fondo Social Europeo for the post-doctoral grant of Trinidad Pérez Palacios and by the FCT grant SFRH/bd/48745/2008 of Catarina Petisca.

REFERENCES

Ansorena D, Gimeno O, Astiasaran I, Bello J. (2001). Analysis of volatile compounds by GC–MS of a dry fermented sausage: chorizo de Pamplona. Food Res Int 34:67–75.

Arts JHE, Muijser H, Appel MJ, Frieke KC, Bessems JGM, Woutersen, RA. (2004). Subacute (28-day) toxicity of furfural in Fischer 344 rats: a comparison of the oral and inhalation route. Food Chem Toxicol 42:1389–1399.

Association of Official Analytical Chemists (AOAC). (1995). Official Methods of Analysis 16th ed. Arlington, VA, USA.

Bastida S, Sanchez-Muniz FJ. (2001). Thermal oxidation of olive oil, sunflower oil and a mix of both oils during forty discontinuous domestic fryings of different foods. Food Sci Technol Int 7:15-23.

Becalski A, Forsyth D, Casey V, Lau BPY, Pepper K, Seaman S. (2005). Development and validation of a headspace method for determination of furan in food. Food Add Cont 22:535–540.

Cerny C, Grosch W. (1992). Evaluatiion of pontent odorants in roasted beef by aroma extract dilution analysis. LWT-Food Sci Technol 194:322-325.

Elmore SJ, Mottram DS, Enser M, Wood JD. (1999). Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. J Agric Food Chem 47:1619-1625

European Food Safety Authority (EFSA). (2009). Furan in heat processed food products including home cooked food products and ready-to-eat products. EFSA-Q-2009-00846.

European Food Safety Authority (EFSA). (2010). Update of results on the monitoring of furan levels in food. The EFSA Journal 8:1702-1720.

Folch J, Less M, Sloane GH. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509.

García C, Berdague JL, Antequera T, Lopez-Bote C, Cordoba JJ, Ventanas J. (1991). Volatile components of dry-cured Iberian ham. Food Chem 41:23–32.

Ho C-T, Lee KN, Jin QZ . (1983). Isolation and identification of volatile flavor compounds in fried bacon. J Agric Food Chem 31:336-342.

Huey K, Abdul S, Mohamed S. (2008. Sensory aroma from Maillard reaction of individual and combinations of amino acids with glucose in acidic conditions. Int J Food Sci Technol 43:1512–1519

International Agency for Research on Cancer (IARC). (1995). Dry cleaning some chlorinated solvents and other industrial chemicals. Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon, France.

ISO 5508. (2000). Animal and vegetable fats and oils—Analysis by gas chromatograph of methyl esters of fatty acids, International Organization for Standardization, Geneva, Switzerland

Jiménez-Martin E, Ruiz J, Pérez-Palacios T, Silva A, Antequera T. (2012). Gas chromatography−mass spectrometry method for the determination of free amino acids as their dimethyl-tert-butylsilyl (TBDMS) derivatives in animal source food. J Agric Food Chem 60:2456-2463.

Mehta U, Swinburn B. (2001). A review of factors affecting fat absorption in hot chips. Crit Rev Food Sci Nutr 41:133–154.

Mellema M. (2003). Mechanism and reduction of fat uptake in deep-fat fried foods. Trends Food Sci Technol 14:364–373.

Mesías-García M, Guerra-Hernández E, García-Villanova B. (2010). Determination of furan precursors and some thermal damage markers in baby foods: ascorbic acid, dehydroascorbic acid, hydroxymethylfurfural and furfural. J Agric Food Chem 58:6027-6032.

Meynier A, Novelli E, Chizzolini R, Zanardi E, Gandemer G. (1999). Volatile compounds of commercial Milano salami. Meat Sci 54:175–183.

Moreira RG, Palau JE, Sun X. (1995). Deep-fat frying of tortilla chips - an engineering approach. Food Technol 49:146–150.

Mottram DS. (1998). Flavour formation in meat and meat products: a review. Food Chem 62: 415-424.

Muriel E, Antequera T, Petrón MJ, Andrés AI, Ruiz J. (2004). Volatile compounds in Iberian dry-cured loin. Meat Sci 68:391-400.

Nawar WW. (1998) Volatile components of the frying process. Grasas y Aceites 49:271-274..

Ozden Z, Erkan N. (2011). A preliminary study of amino acid and mineral profiles of important and estimable 21 seafood species. British J Food 113:457-469.

Pérez-Palacios T, Petisca C, Melo A, Ferreira IMPLVO. (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 Chem 135:1337-1343.

Pérez-Palacios T, Ruiz J, Martín D, Grau R, Antequera T. (2010). Influence of pre-cure freezing on the profile of volatile compounds during the processing of Iberian hams. J Sci Food Agric 90:882-890.

Pinthus EJ, Weinberg P, Saguy IS (1993) Criterion for oil uptake during deep-fat frying. J Food Sci 58:204-205.

Ramírez R, Estévez M, Morcuende D, Cava R. (2004). Effect of the type of frying culinary fat on volatile compounds isolated in fried pork loin chops by using SPME-GC-MS. J Agric Food Chem 52:7637-7643.

Romero A, Cuesta C, Sanchez-Muniz FJ. (2000). Cyclic fatty acid monomers and thermoxidative alteration compounds formed during frying of frozen foods in extra virgin olive oil. J Am Oil Chem Soc 77:1169-1173.

Ruiz J, Muriel E, Ventanas J. (2002). The flavour of Iberian ham. In Toldrá F, ed. Research advances in the quality of meat and meat products. Trivandrum, India: Research Signpost, 255-271.

Saguy SI, Dana D. (2003). Integrated approach to deep fat frying: engineering, nutrition, health and consumer aspects. J Food Eng 56:143-152.

Sánchez-Muniz FJ, Viejo JM, Medina R. (1992). Deep-frying of sardines in different culinary fats. Changes in the fatty acid composition of sardines and frying fats. J Agric Food Chem 40:2252-2256.

Shahidi F, Rubin LJ, D'Souza L. (1986). Meat flavour volatiles: a review of the composition, techniques of analysis, and sensory evaluation. Crit Rev Food Sci Technol 24:141–243.

Southern CR, Chen XD, Farid MM, Howard B, Eyres L. (2000). Determining internal oil uptake and water content of fried thin potato crisps. Food and Bioprod Processing 78:119–125.

Stahnke LH, Holck A, Jensen A, Nilsen A, Zanardi E. (2002). Maturity acceleration of Italian dried sausage by Staphylococcus carnosuss relationship between maturity and flavour compounds. J Food Sci 67:1914-1921.

Sujatha PS. (2008). Monitoring cytotoxic potentials of furfuryl alcohol and 2-furyl methyl ketone in mice. Food Chem Toxicol 46:286-292.

Swasti YR, Murkovic M. (2011). Characterisation of furfuryl alcohol polymerisation in roasted coffee. In Proceedings of 1st International Congress on Cocoa, Coffee and Tea. Novara, Italy.

Timón ML, Carrapiso AI, Jurado A, Lagemaat J. (2004). A study of the aroma of fried bacon and fried pork loin. J Sci Food Agric 84:825-831.

Ufheil G, Escher F. (1996). Dynamics of oil uptake during deep-fat frying of potato slices. LWT-Food Sci Technol 29:640–644.

US Department of Health and Human Service. (2005). 11th Report on Carcinogens Public Health Service, National Toxicology Program, Research triangle park, NC.

Wagner RK, Grosch W. (1998). Key odorants of French fries. J Am Oil Chem Soc 75:1385-1392.

Figure 1. Families of chemical volatile compounds (expressed as percentage of total chromatographic area) in non-fried (□) and deep fried (■) coated fish products.

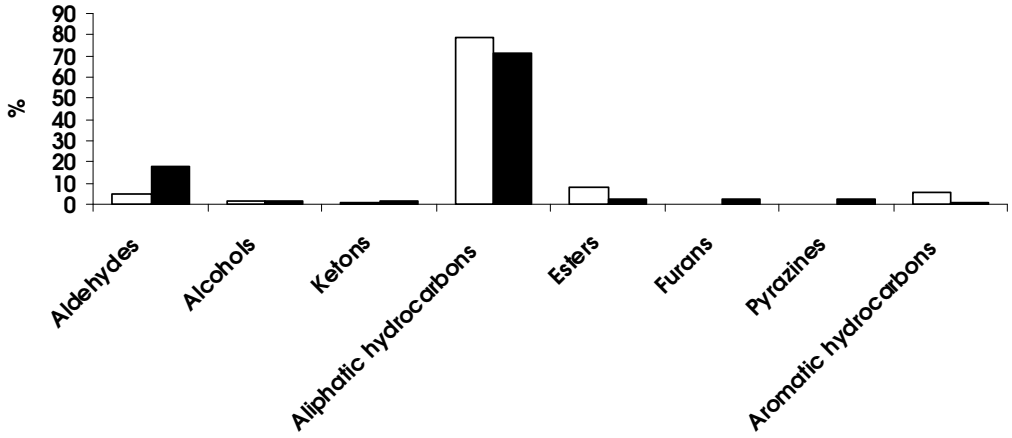

Table 1. Fatty acid composition (g FAME per 100 g FAME detected) in non-fried (NF) and deep-fried (F) coated fish products.

	NF	F	*p*
C16:0	8.20 ± 0.52	7.02 ± 0.37	0.001
C16:1 n-7	0.34 ± 0.24	0.32 ± 0.09	0.807
C18:0	3.61 ± 0.29	3.43 ± 0.43	0.604
C18:1 n-9	40.75 ± 0.61	33.37 ± 0.90	< 0.001
C18:2 n-6	42.51 ± 1.79	52.45 ± 1.24	< 0.001
C18:3 n-6	0.35 ± 0.01	0.01 ± 0.00	< 0.001
C18:3 n-3	1.55 ± 0.25	0.36 ± 0.27	< 0.001
C22:0	0.79 ± 0.09	0.86 ± 0.05	0.163
C20:4 n-6	0.13 ± 0.07	0.09 ± 0.03	0.286
C20:5 n-3	0.23 ± 0.07	0.23 ± 0.04	0.981
C24:0	0.33 ± 0.05	0.52 ± 0.12	0.007
C22:6 n-3	1.43 ± 0.71	1.46 ± 0.27	0.928
SFA	12.95 ± 0.78	11.95 ± 0.77	0.113
MUFA	40.80 ± 0.67	33.48 ±0.87	< 0.001
PUFA	45.95 ± 1.10	54.36 ± 1.12	< 0.001
∑ n-6	42.98 ± 1.71	52.54 ± 1.23	< 0.001
∑ n-3	3.25 ± 0.63	2.12 ± 0.25	0.005
∑ n-6/n-3	13.71 ± 2.07	24.86 ± 2.31	< 0.001

Table 2. Amino acid composition (mg per g dry matter of edible food) in non-fried (NF) and deep-fried (F) coated fish products.

	NF	F	*p*
Alanine	0.42 ± 0.01	0.50 ± 0.11	0.256
Glycine	0.40 ± 0.01	0.49 ± 0.05	0.045
Valine	0.41 ± 0.02	0.37 ± 0.01	0.026
Leucine	0.14 ± 0.00	0.14 ± 0.01	0.153
Isoleucine	0.15 ± 0.00	0.13 ± 0.01	0.021
Proline	0.35 ± 0.00	0.33 ± 0.01	0.095
Methionine	0.30 ± 0.01	0.26 ± 0.01	0.004
Threonine	nd	2.16 ± 0.36	-
Phenylalanine	2.96 ± 0.08	13.04 ± 2.24	0.001
Aspartic acid	3.22 ± 0.19	6.14 ± 0.26	< 0.001
Cysteine	19.15 ± 0.13	46.01 ± 7.39	0.003
Glutamic acid	0.08 ± 0.01	nd	-
Arginine	0.37 ± 0.01	0.31 ± 0.04	0.088
Asparagine	0.47 ± 0.01	0.39 ± 0.01	0.001
Lysine	0.37 ± 0.01	0.42 ± 0.06	0.155
Glutamine	0.40 ± 0.01	0.45 ± 0.03	0.048
Histidine	0.39 ± 0.01	0.41 ± 0.03	0.195
Tyrosine	0.08 ± 0.00	0.13 ± 0.00	< 0.001
Triptophan	0.55 ± 0.01	0.78 ± 0.08	0.007
∑AA	30.20 ± 0.22	72.47 ± 10.69	0.001

nd, not detected

Table 3. Abundance (UA x 10^7) of volatile compounds detected in non-fried (NF) and deep-fried (F) coated fish products.

	RI[a]	ID[b]	NF	F	*p*
ALDEHYDES					
2-methylpropanal	546	AB	nd	2.05	-
3-methylbutanal	652	AB	nd	4.45	-
2-methylbutanal	662	AB	nd	4.08	-
hexanal	798	AB	11.32	27.78	0.131
2-hexenal	853	AB	nd	1.17	-
Heptanal	901	AB	1.41	3.97	0.049
2-heptenal	654	AB	1.53	11.53	0.067
benzaldehyde	968	AB	2.31	2.74	0.625
octanal	1003	AB	1.15	1.75	0.307
benzeneacetaldehyde	1050	AB	0.70	1.69	0.047
2-octenal	1060	AB	nd	3.00	-
nonanal	1107	AB	3.51	7.13	0.066
nonenal	1170	AB	0.57	0.81	0.117
2-decenal	>1200	A	0.62	2.82	0.012
2,4-decadienal	>1200	A	5.28	23.77	0.012
2-dodecenal	>1200	A	0.32	1.86	0.004
Total aldehydes			29.80	101.99	0.040
ALCOHOLS					
2-methyl-1-butanol	733	AB	1.65	nd	-
1-pentanol	765	AB	0.75	2.90	0.103
2-pentanolacetate	847	A	1.19	nd	-
1-hexanol	867	AB	1.35	nd	-
1-butanol-3-methyl, acetate	873	A	2.92	nd	-
1-octen-3-ol	979	AB	2.10	3.91	0.070
2-ethylhexanol	1029	AB	2.06	0.65	0.112
Total alcohols			12.03	7.46	0.096
KETONS					
2-pentanonone	685	AB	4.46	4.06	0.458
2,3-pentanedione	695	A	nd	2.41	-
2-heptanone	890	AB	nd	0.82	-
Total ketons			4.46	7.97	0.039
ALIPHATIC HYDROCARBONS					
2-methylpentane	555	AB	211.79	222.47	0.913
3-methylpentane	576	AB	71.04	71.96	0.980
hexane	600	AB	225.75	112.95	0.203
heptane	700	AB	11.92	nd	-
2-octene	805	AB	nd	0.86	-
decane	1000	AB	3.47	1.32	0.139
undecane	1100	AB	0.92	nd	-
dodecene	1292	AB	nd	0.44	-
dodecane	1200	AB	1.27	nd	-
Total aliphatic hydrocarbons			516.16	412.00	0.536

Table 1. (*Continued*)

	RI[a]	ID[b]	NF	F	*p*
ESTERS					
acetic acid, ethyl ester	613	AB	24.89	10.23	0.113
propanoic acid, methyl ester	628	A	3.22	0.96	0.065
butanoic acid, methyl ester	721	A	3.46	2.67	0.201
butanoic acid, 2-methylprotylester	954	A	1.67	nd	-
butanoic acid, butyl ester	993	A	7.50	nd	-
acetic acid, hexil ester	1100	AB	1.43	nd	-
butanoic acid, 3-methyl, butyl ester	1055		4.75	nd	-
octanoic acid, methyl ester	1130	A	1.65	nd	-
butanoic acid, hexyl ester	1129	A	2.48	nd	-
octanoic acid, ethyl ester	1196	A	0.76	nd	-
Total esters			52.56	13.85	0.002
FURANS					
furan	512	AC	nd	0.47	-
furfural	833	ABC	nd	3.43	-
furfurylalcohol	854	ABC	nd	2.86	-
2-pentylfuran	993	ABC	nd	7.61	-
Total furans			-	14.96	-
PYRAZINES					
methylpyrazine	824	AB	nd	7.92	-
dimethylpyrazine	913	AB	nd	3.23	-
ethylpyrazine	918	A	nd	0.83	-
Total pyrazines			-	11.98	-
AROMATIC HYDROCARBONS					
benzene	661	A	1.18	nd	-
methylbenzene	786	AB	12.90	2.89	0.128
chlorobenzene	850	A	0.84	nd	-
1,3-dimethylbenzene	873	AB	1.11	1.48	0.752
ethenylbenzene	895	AB	1.14	0.89	0.466
1-ethyl,3,5-dimethylbenzene	1032	A	1.48	nd	-
limonene	1037	AB	18.74	0.96	0.002
naphthalene	>1200	A	0.40	nd	0.166
Total aromatic hydrocarbons			37.60	6.12	0.031

[a] RI, retention index

[b] ID, method of identification: A, tentative identification by mass spectrum; B, RI in accordance with literature; C, mass spectrum and RI identical to a reference compound and *m/z* ion.

nd, not detected