An investigation of process contaminants' formation during the deep frying of breadcrumbs using a bread coat model

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ABSTRACT

The formation of acrylamide, hydroxymethylfurfural (HMF) and furfural was investigated in a deep fried breadcrumb coat model resembling the coat batter of breaded foods. The influence of the composition of the breadcrumb and the frying conditions on the formation of these contaminants was evaluated. Six wheat-based flour formulations of breadcrumbs were deep fried in sunflower oil at temperatures between 170–200 °C and for frying times of 1–5 minutes. Results showed significant differences in the levels of contaminants according to the concentration of the potential precursors in the breadcrumbs. HMF was influenced by the sugar content in the breadcrumbs whereas levels of acrylamide were significantly correlated with the ratio between asparagine and reducing sugars. Acrylamide, HMF and furfural were directly related to the frying time and temperature. The composition of the breadcrumb and the compounds formed during frying contributed to the total antioxidant capacity of the fried samples. The bread coat model is a useful tool in the formulation of breaded foods since it allows the evaluation of the contribution of breadcrumbs in the formation of process contaminants after frying.

INTRODUCTION

Dietary habits have changed over the last few years, with an increase in the consumption of processed foods such as snacks and fast food and a decrease in the consumption of natural foods.[1] The changes in the current society (e.g. working conditions, socioeconomic trends, lack of time for food preparation) make processed foods a common choice for everyday meals at a moderate cost.[2] These eating habits include consumption in fast food establishments and food services, but the household demand for quick and convenient meal solutions is also increasing over years. In Spain, eating habits are moving away from the Mediterranean diet with a higher incidence in the population below 45 years.[3] For example, consumption of convenience foods increased from 23.3 to 34.2 g per person per day in the period 2000–2006.[1]

Batter and breadcrumb coating processes are conventionally used for a variety of food products which are subjected to deep frying. Such food products usually comprise sea foods, poultry, red meats and vegetables, but a variety of other food products may also be desirably batter and bread coated to provide an aesthetically appealing food product to consumers. The outer layer contributes to the properties of the product as it forms a crust which is more attractive once fried. In addition, it can control moisture loss and oil uptake, thus improving the nutritional quality of the product.[4] The negative aspect of the consumption of these foods is primarily related to the high energy content, which is linked to an increase in obesity and other health concerns[4] but moreover, an emerging risk is also associated due to a high exposure to Maillard reaction products (MRPs).[5]

Breaded foods are subjected to deep frying in vegetable oils at high temperature, where the Maillard reaction takes place with the resulting formation of process contaminants, such as acrylamide, hydroxymethylfurfural (HMF) and furfural.[6,7] Acrylamide is generated as a result of the reaction between asparagine and reducing sugars as main precursors. Recently, the European Food Safety Agency has confirmed that the presence of acrylamide in food is a public health concern, requiring continued efforts to reduce its exposure.[8] In a similar way, HMF and furfural are formed as intermediate products of the Maillard reaction and furthermore, HMF is also generated by the caramelization of sugars at high

temperature.[9,10] Based on studies in animals, HMF is suspected to have genotoxic and mutagenic effects through its metabolism product sulphoxymethylfurfural[11,12] whereas furfural may lead to hepatotoxicity.[13]

The formation of process contaminants in breaded foods is mainly located in the crust and their occurrence depends on many factors, although time and temperature are two important process parameters affecting their formation during frying.[14] Since breaded products are widely consumed, process contaminants from these products significantly contribute to human daily exposure and thus, it is a public health concern. Several studies have evaluated the process contaminants' formation in completely breaded foods, such as breaded chicken[6] and breaded fish.[7] In these cases, the water content of the product and the interaction between the ingredients present in the product and those present in the coat may influence the formation of the contaminants. However, to the best of our knowledge, little information is available on the specific contribution of breadcrumbs in the formation of process contaminants, since previous investigation has been carried out using the whole food. Thus, a bread crust model has been developed to resemble the crust of breaded foods with the aim to investigate the formation of acrylamide, HMF and furfural in deep fried breadcrumbs and its relationship with the composition of the breadcrumbs. The influence of the time and temperature of cooking on the contaminant formation was also evaluated.

MATERIALS AND METHODS

Reagents and chemicals

Ethylenediaminetetraacetic acid (EDTA), Folin–Ciocalteu reagent, ammonium acetate, and sodium bicarbonate were obtained from Panreac (Madrid, Spain). 2,2'-Azobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was purchased from Fluka Chemical (Madrid, Spain). Acrylamide standard (99%), D(+)-glucose, phenyl isothiocyanate (PITC), gallic acid, HMF, furfural, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and potassium persulfate were purchased from Sigma (St Louis, MO, USA). Acetonitrile, formic acid, potassium ferrocyanide (Carrez-I), zinc acetate (Carrez-II), asparagine and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). [$^{13}C_3$]-acrylamide (isotopic purity 99%) was from Cambridge Isotope Labs (Andover, MA, USA). Milli-Q water used was produced using an Elix 3 Millipore water purification system coupled to a Milli-Q module (model Advantage A10) (Millipore, Molsheim, France). All other chemicals, solvents and reagents were of analytical grade.

Samples

Six wheat-based flour breadcrumb (BC) samples were supplied by three national producers. Four samples (BC-2, BC-3, BC-4, BC-6) were ready-to use for consumers, and two samples (BC-1, BC-5) were designed for coating par-fried breaded products in the food industry. Samples BC-1, BC-2, BC-3 and BC-5 were formulated with wheat flour, water, salt and yeast; sample BC-4 also contained wheat gluten, olive oil and a raising agent; sample BC-6 also contained garlic and parsley. Sample BC-4 was ground additionally to obtain a typical medium particle size similar to other samples (~1–2 mm). However, specific information about the industrial processing conditions applied for kneading, fermentation and baking is not available.

Description of the bread model for frying

1.5 g of the sample was placed in a stainless steel bag (6 × 7 cm, mesh of 0.20 mm, thickness 2 mm) as depicted in Fig. 1. The bag had a mesh wide enough to allow the oil absorption and water evaporation during frying, but small enough to retain the sample within the bag. Breadcrumbs were distributed homogeneously in a thin layer. The aim was to mimic the coat of a breaded product, with a uniform distribution and suitable thermal transference regardless of the effect of the food matrix. Samples were deep fried in sunflower oil at 180 °C for 3 minutes using a domestic fryer (Jemi, Barcelona, Spain, 5 L

capacity, 2200 W). Reference frying conditions of temperature and time were adjusted according to industrial recommendations. A calibrated K-type thermocouple data logger (Delta Ohm, Caselle di Selvazzano, Italy) was used to monitor the temperature during frying. The frying started 10 minutes after the oil temperature reached the target value and stabilized. For the kinetic studies, breadcrumbs (n = 3) were fried under different conditions of time and temperature according to (a) different times at a constant temperature (1, 2, 3, 4 and 5 min at 180 °C), and (b) different temperatures at a constant time (170, 180, 190 and 200 °C for 3 min). Eighteen bags per sample were fried in each combination of time/temperature.

Validation of the bread model for frying

To validate the frying model sample, BC-5 was taken as a reference and fried at 180 °C for 3 min. The precision of the model was evaluated through analysis on the same day (repeatability) and on different days (reproducibility). Nine replicates were analyzed for each assay. The parameters evaluated were colour, HMF and acrylamide contents.

Direct determination of polar compounds in oil

Total polar compounds were measured after each cycle of frying by a hand-held device Testo 270 (Testo INC, New Jersey, USA). Results were expressed as percentage of the total polar material.

Determination of moisture

Moisture was determined gravimetrically to a constant weight in an oven (Memmert, Schwabach, Germany) at 105 °C for 24 h according to the AOAC method.[15]

Determination of water activity (A_w)

The water activity of breadcrumbs and fried breadcrumbs was measured by an AquaLAB CX-2 (Decagon Devices Inc., Pullman, WA).

Measurement of pH

Breadcrumbs (1 g) were mixed with 100 mL of water and vortexed for 3 min. The mixture was held at room temperature for 1 h to separate the phases. After carefully removing the supernatant layer, the pH was measured using a CG-837 pH meter (Schott, Mainz, Germany).

Determination of oil uptake by NMR spectrometry

Oil uptake in the fried samples was measured using a low resolution NMR spectrometer, the Minispec mq-20 (Bruker Optics, Ettlingen, Germany) equipped with a 40 °C permanent magnet of 0.47 T and operating at 20 MHz operating frequency. Measurements were made in 15 mm diameter glass tubes. Firstly the device was calibrated with 5 quantities of sunflower oil (from 0.2 to 1.0 g) and a calibration line was built between NMR responses and the corresponding oil weights (ISO 5511, 1992). The NMR procedure was validated with the conventional Söxhlet extraction procedure. A quantity of fried breadcrumb sample (W_{sample}) was placed in a glass tube, to a maximum height of 2 cm. The oil weight of the sample (W_{oil}) was measured by NMR. Oil uptake was then determined by the software using the formula:

$$\text{Oil uptake} = W_{oil} / W_{sample} \times 100$$

Determination of the total protein content

The total protein content was determined in samples using an automated nitrogen analyzer (FP-2000; Dumas Leco Corp., St Joseph, MI), after calibration of the instrument with EDTA. The nitrogen-to-protein conversion factor was N × 5.70. The results were expressed as g of protein per 100 g of the product.

Determination of reducing sugars

The reducing sugar content was determined by Miller.[16] A calibration plot was drawn using a standard glucose solution in the range of 0.25 to 2.0 mg mL^{-1}. Results were expressed as mg glucose equivalents per g of the sample. The limit of quantification was set at 25.2 mg glucose equivalents per g of the sample.

Determination of free asparagine

Free asparagine was extracted from the breadcrumb samples with 0.1 N HCl. Derivatization by using PICT and HPLC quantification was carried out following the method of Martínez-Villaluenga et al.[17] A Shimadzu HPLC system (Kyoto, Japan) equipped with an LC-20AD pump, an SIL-10ADVP auto autosampler, a CTO-10ASVP oven and an SPD-M20A diode array detector was used. The sample (5 μL) was injected into a Kinetex (100 × 4.6 mm, 2.6 μm, Phenomenex, Torrance, CA, USA). A gradient mixture of 0.1 M ammonium acetate (A) and 0.1 M ammonium acetate : methanol : acetonitrile (B) at a flow rate of 1 mL min^{-1} at 43 °C was used. The eluent composition started with 100% of A, decreased to 90% in 4 min, to 70% in 8 min, to 50% in 10 min, to 0% in 13 min and held until 14 min. Then, it was linearly increased to its initial conditions (100% of A) at 15 min and the total chromatographic run was completed in 25 min. An asparagine standard was used for the calibration and norleucine was used as an internal standard. Results were expressed as mg per 100 g of the product.

Colour determination

The measurements were performed using a HunterLab Spectrophotometer CM-3500D colorimeter (Hunter Associates laboratory, Stamford, Connecticut, USA). Three independent measurements of a*(redness), b*(yellowness) and L*(lightness) parameters were carried out on different areas of the breadcrumb and fried breadcrumb samples. The E index was calculated according to the following equation: $E = (L^2 + a^2 + b^2)^{1/2}$, which allows evaluating the colour changes in the samples.

Determination of the total antioxidant capacity by direct ABTS+ assay

The direct measurement of the total antioxidant activity by the QUENCHER approach was performed according to Gökmen et al.[18] and adapted to a microplate reader. 5 mg of both breadcrumb and fried breadcrumb samples were mixed with 4 mL of ethanolic solution (50%) and 1 mL of dilute $ABTS^{+}$ solution (7 mM). An absorbance reading was taken at 40 min using a Synergy™ HT-multimode microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA). Trolox was used for the calibration and results were expressed as μmol equivalents of Trolox (TEAC) per g of the sample.

Determination of Folin–Ciocalteu (FC) reducing capacity

The Folin–Ciocalteu reducing capacity of breadcrumbs and fried breadcrumbs was determined as described by Singleton et al.[19] and adapted to a plate-reader. A direct measurement of the samples without extraction prior to analysis was carried out following the method of Horszwald et al.[20] Absorbance of the samples was recorded using a Synergy™ HT-multimode microplate spectrophotometer as described above and quantified using gallic acid as a standard. Results were expressed as μg of gallic acid equivalents (GAE) per g of the sample.

Determination of HMF and furfural

HMF and furfural were determined following the HPLC method described by Rufián-Henares et al.[21] The limit of quantification was set at 0.6 mg kg^{-1} and 0.3 mg kg^{-1} for HMF and furfural, respectively. Analyses were done in duplicate and results were expressed as mg per kg of the product.

LC-ESI-MS-MS determination of acrylamide

Acrylamide was determined as described by Mesías et al.[22] The limit of quantitation was set at 16 μg kg^{-1}. The accuracy of the results was recently demonstrated for crispbread in an interlaboratory

comparison study launched by the Food Analysis Performance Assessment Scheme (FAPAS) program (2015), yielding a z-score of 0.3. Results were expressed as μg per kg of the product.

Statistical analysis

Statistical analyses were performed using Statgraphics Centurion XV (Herndon, VA, USA). Analysis was performed at least in duplicate. Data were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) and the least significant difference (LSD) test were applied to determine differences between means. Differences among samples and the effect of the frying process were evaluated. Differences were considered to be significant at $p < 0.05$. Relationships between the different parameters analyzed were evaluated by computing Pearson linear correlation coefficients at the $p < 0.05$ confidence level.

RESULTS AND DISCUSSION

Validation of the frying model

A bread coat frying model was developed to investigate the specific influence of the breadcrumbs on the formation of acrylamide, HMF and furfural during deep frying. This coat frying model is just focused on the coat of a breaded food and subsequently no influence of the food matrix on the behaviour of the breadcrumbs during frying is considered. Therefore, matrix effects on the moisture or the interaction with food ingredients, which have been demonstrated to influence the formation of process contaminants in breaded products,[6,23] are not taken into account in the present study. In the first step, the precision of the model was evaluated. Sample BC-5 was placed in nine different stainless steel bags and fried at 180 °C for 3 min (3 bags per trial) on the same day (repeatability assay) and on different days (reproducibility assay). Colour parameters, HMF and acrylamide were determined in each bag of fried breadcrumbs. The relative standard deviations (RSD) for both repeatability and reproducibility experiments were 3.3% and 3.5% for a*, 4.1% and 4.3% for b*, 3.6% and 3.6% for L*, 9.0%, and 11.0% for HMF and 7.4% and 6.8% for acrylamide. As RSD was mainly lower than 12%, the bread coat model was considered robust.

Characterization of the breadcrumb and fried breadcrumb samples

BC samples were characterized to estimate the content of potential precursors and parameters that may play an important role in the progression of the Maillard reaction, and subsequently to contribute to the formation of process contaminants. Moisture, water activity, pH, protein, reducing sugar content, free asparagine and colour are summarized in Table 1. Moisture and water activity showed mean values of 7.0 ± 0.7% and 0.34 ± 0.04, respectively, BC-2 and BC-5 being the lowest and the highest, respectively. Samples presented similar pH values with the exception of BC-1 with a pH value of 7.8. The protein content ranged between 9.2 (BC-5) and 12.7 g per 100 g (BC-4) and the reducing sugar content between 2.1 (BC-1) and 6.7 g per 100 g (BC-6). The highest variability was found in the asparagine content probably related not only to the composition of the flours, but also influenced by the manipulation conditions of the doughs;[24] thus values ranged from 3.2 to 25.3 mg per 100 g in BC-2 and BC-6, respectively. The colour was similar among the samples and ranged from 73.4 (BC-6) to 81.6 (BC-4). The differences in colour cannot be further explained in terms of the baking conditions applied since this information was not provided by the manufacturer. These results cannot be directly compared with bread since bread is distinguished for having a crunchy and golden-yellow crust, spongy and light crumbs with a soft texture and intermediate moisture. However, the baking conditions for breadcrumbs are driven to maximise the formation of the crust as observed in their relatively low moisture content. Similar results have been reported by Ahrné et al.[25] in baked wheat bread. Samples were further characterized by their total antioxidant capacity (radical scavenging and reducing capacity). Although the Folin–Ciocalteu assay is the most popular method to evaluate the total phenolic compounds, the Folin–Ciocalteu reagent can be reduced by many electrondonors, and not only by phenolic compounds such as those formed during the Maillard reaction.[26] The total antioxidant capacity ranged from 10.1 (BC-1)

to 23.8 μmol TEAC per g (BC-3), and the FC reducing capacity ranged from 20.9 (BC-5) to 46.2 μg GAE per g (BC-2). A similar antioxidant capacity has been reported by other authors.[27,28]

HMF, furfural and acrylamide were analyzed in the breadcrumbs. Acrylamide was not detected; however the HMF and furfural content varied within a wide range. The HMF content ranged from not being detected (BC-1) to 19.4 mg kg− 1 (BC-3), and furfural ranged from not being detected (BC-1, BC-4) to 1.4 mg kg− 1 (BC-3). A median value of 15 μg kg^{-1} has been indicated for acrylamide in wheat soft bread (mean value: 38 μg kg− 1)8 and up to 19.1 mg kg^{-1} for HMF in wheaten bread.[13]

Samples were deep fried in the coat model at 180 °C for 3 minutes as recommended by the manufacturer. The content of polar compounds in the frying oil was measured after each cycle, obtaining levels lower than 5%. Frying oil will be replaced above this threshold.[29] The formation of process contaminants increased drastically after frying and differences between samples became significant (Table 1). The HMF content ranged from 7.8 to 95.5 mg kg^{-1}, furfural from 3.7 to 10.4 mg kg^{-1} and acrylamide from 78 to 538 μg kg^{-1}. These results cannot be compared with the previous results in the literature since they are related to the whole breaded food and are not specific to the contribution of the coat layer. These variations demonstrate that the composition and the type of breadcrumb used are the key factors to promote or minimize the formation of process contaminants during deep-frying of breaded food.

It is well known that the formation of the MRPs directly depends on the content of amino acids and reducing sugars and, in addition, on the pH, moisture and water activity of the food.[30] In the present study, samples with the lowest (BC-1) and the highest (BC-2) reducing sugar content displayed the lowest and the highest levels of HMF after frying, which presumably was formed through both sugar caramelization and the Maillard reaction. However, sample BC-2 reached nearly three-fold the content of HMF as compared with BC-3. It could be plausible that precursors other than reducing sugars are present in the formulation, like a leavening agent or even different concentrations in sodium chloride. In addition, a measurement of the reducing sugar does not discriminate between fructose and glucose. Fructose is more reactive than glucose in the formation of HMF through direct dehydration,[31] and in a dry system HMF also is formed from sucrose through a very reactive fructofuranosyl cation.[32] Then, the presence of these precursors in the breadcrumb formulation could explain the greater difference in HMF between samples BC-2 and BC-3 after deep frying.

In contrast, acrylamide formation is highly influenced by the ratio of reducing sugars (RS) and asparagine (ASN) in the sample. This statement is observed in Fig. 2. BC-2 that contains the lowest ASN/RS ratio generated the lowest level of acrylamide and sample BC-1 with the highest ratio generated the highest amount of acrylamide. Our results show that the use of wheat flour with a low asparagine content for the formulation of breadcrumbs resulted in a lower formation of acrylamide after frying. In agreement with Amrein et al.,[33] this result confirms that asparagine may be considered the limiting factor for acrylamide formation in cereal products. In addition, the statement of the acrylamide toolbox[34] that asparagine is a key determinant for cereal/grain based products like bread, crispbread, breakfast cereals and biscuits could be extended to cereals under frying conditions and not only under baking.

Variations in the moisture content and pH values influence the extent of the Maillard reaction.[30] However correlations between these parameters and the formation of the chemical contaminants were not observed, except for furfural, whose formation was negatively correlated with the moisture content and water activity in the initial breadcrumbs (r^2 = − 0.8703, p = 0.0241; r^2 = − 0.8408, p = 0.0360, respectively). As expected, the moisture content and water activity of breadcrumbs decreased after the frying process, showing values significantly lower than those of the initial breadcrumbs (56–81% and 19–72%, respectively), which in turn are associated with the progression of the Maillard reaction. Significant

correlations were not found between the protein content and the formation of process contaminants. Regarding colour parameters, a decrease in the lightness values (L*) and an increase in the redness values (a*) of the fried breadcrumbs with the frying process lead to a significant decrease in the E index as a consequence of the Maillard reaction and caramelization,[14] although these parameters were not significantly correlated. Similar results have been reported by Ngadi et al.[35] when the authors related colour parameters of fried chicken nuggets with the frying time.

One of the main problems associated with bread coated food consumption is the considerable amount of oil absorbed during the frying operation.[4] Oil uptake in fried breadcrumbs ranged from 27.5 to 35.7% as summarized in Table 1.

The changes in the antioxidant capacity are summarized in Table 1. BC-1 and BC-4 significantly increased their radical scavenging capacity, BC-2, BC-3 and BC-5 significantly decreased it and BC-6 showed no significant changes. In contrast, values for the reducing capacity of the breadcrumbs, as recorded by the FC assay, increased significantly after frying. This effect is due to the formation of Maillard reaction products with antioxidant capacity[20] but, in addition, because of the contribution of the tocopherols and polyphenols in the sunflower oil.[36] The input of new compounds, however, was not enough to increase the total antioxidant properties in BC-2, BC-3 and BC-5, where the degradation of the compounds with antioxidant capacity present in the non-fried breadcrumbs prevailed over the contribution of those generated during frying. The unchanged levels for the radical scavenging capacity of BC-6 could be explained by the balance resulting from the degradation of compounds already present in the commercial samples and the contribution of the new products formed. BC-6 contained garlic and parsley added to the breadcrumbs after baking in the factory, whose antioxidant capacity may make it different from the others after the frying process.[37,38] Similar results were shown by Horszwald et al.[20] These authors indicated that some antioxidants naturally present in flour or produced during dough preparation of rye bread were degraded during baking, while others, such as MRPs, were newly generated during the formation of the crust. Similarly, Žilić et al.[39] have recently reported that baking conditions reduced the content of anthocyanins and total flavonoids in cookies and increased the content of free phenolic acids released from the dietary fibers. This increase together with the formation of MRPs contributed to greater values of the total antioxidant capacity. Significant correlations between the formation of process contaminants and the antioxidant capacity were not found in the fried breadcrumbs.

Kinetic experiments: effect of frying time and temperature

Three of the four basic formulations of breadcrumbs (wheat flour, water, salt and yeast) showing the highest (BC-1), the lowest (BC-2) and intermediate (BC-5) levels of acrylamide after the frying process in the bread coat model were selected for the following set of experiments. Samples were subjected to two kinetic studies where the effect of time and temperature on the formation of process contaminants during frying was evaluated. The conditions used were those according to the industrial recommendations.

Changes in the moisture, water activity, colour and oil uptake during the kinetic experiments followed the same trend as that explained before during the frying process, without a significant impact of the frying time and temperature.

Changes in the process contaminant content in breadcrumbs fried under different conditions are shown in Fig. 3 and 4. The results clearly show that both the frying time and temperature have strong influences on HMF, furfural and acrylamide formation in fried breadcrumbs. For HMF and furfural, the greatest formation was shown between 2–3 minutes, especially in BC-2 (Fig. 3), which progressively continued to

increase until reaching concentrations of 103.4 mg kg^{-1} at 5 minutes for HMF. In contrast, BC-1 and BC-5 seemed to reach the upper level at 4 minutes, showing the final values of 17.0 and 39.4 mg kg^{-1} for HMF and 6.8 and 5.2 mg kg^{-1} for furfural. In parallel, both HMF and furfural regularly increased with increasing frying temperatures (Fig. 4), BC-2 again displaying the highest concentrations. In this experiment, levels of HMF were 50.4, 140.0, 86.9 mg kg^{-1} for BC-1, BC-2 and BC-5, respectively, when the frying temperature was 200 °C. Under these conditions furfural levels were 14.3, 14.7, 10.7 mg kg^{-1}. These values exceeded those at 5 minutes of frying and surpassed 4–14 times the concentrations of HMF and 2–6 times that of furfural when frying at 170 °C. Regarding acrylamide, BC-2 did not exhibit a significant increase during the frying time whereas in BC-1 and BC-5 it increased during the 5 minutes, most notably between the first and second minute (Fig. 3). Açar and Gökmen[40] also observed an increase of acrylamide formation with time for the process in which the crust model was subjected to baking. When temperatures were tested the raise was more progressive, BC-1 showing the highest values, BC-5 intermediate values and BC-2 the lowest ones (Fig. 4). Again, results reached at 200 °C were higher than those at 5 minutes except for BC-1, which came to the plateau at 190 °C. These findings are in concordance with those found in the literature.[41]

The degree of formation of the chemical contaminants after frying could be related to the precursor levels in the breadcrumbs. In this regard, BC-1, with the lowest content of reducing sugars and a high content of asparagine, showed the lowest levels of HMF and the highest ones for acrylamide. In contrast, BC-2 with the highest content of reducing sugars and the lowest content of asparagine showed the highest levels of HMF and the lowest ones for acrylamide. Despite BC-5 containing high concentrations of asparagine and reducing sugars, the levels of contaminants were intermediate, which means that other factors may be involved in the formation of these products. The moisture content, water activity and pH could also be involved in the reaction. BC-5, specifically, presented the highest pH value, which maybe attenuated by the formation of the contaminants. According to the manufacturer, the composition of the 3 commercial breadcrumbs is similar although the amount of the different ingredients is not specified. The salt content affects the formation of MRPs, decreasing acrylamide and increasing HMF formation.[22] Therefore its influence on the process contaminants' development cannot be discarded. On the other hand, acrylamide can also be formed from frying oil but, in agreement with previous research, this contribution has not been considered.[42]

Changes in the antioxidant capacity with the frying time and temperature were also evaluated by direct measurements of the breadcrumbs (Fig. 5 and 6). Both ABTS and FC reducing capacity assays indicate that the antioxidant capacity progressively increased with the frying time and temperature, associated with the development of the Maillard reaction. The three samples followed similar trends except for the result of ABTS in BC-2 and BC-5 during the first frying minute. In this case thermolabile compounds providing antioxidant capacity were degraded at the beginning of the frying process. As the precise formulation of the breadcrumbs was not provided by the manufacturer, this statement cannot be further clarified.

CONCLUSIONS

In summary, these experiments conclude that similar wheat based flour formulations of breadcrumbs deep fried under the same conditions result in different levels of chemical process contaminants and different antioxidant capacities. The composition of the breadcrumbs as an ingredient of breaded foods will largely impact the formation of acrylamide, HMF and furfural during the deep frying process. A particularly high asparagine/reducing sugar ratio promotes a higher formation of acrylamide under the same frying conditions. Therefore, not only are the frying conditions critical, so are the ingredients used in the breadcrumb formulation to minimize the formation of potentially harmful compounds in the crust of breaded products. Taking into account these experimental data, it should be possible to manufacture breaded products with reduced levels of acrylamide, HMF and furfural by controlling both the

composition of the product and the frying parameters and, subsequently, reducing their contribution to the dietary intake of the population. In this sense, the bread coat model designed in this investigation may be a useful tool in the formulation of breaded foods since it allows the estimation of the contribution of breadcrumbs in the formation of process contaminants after frying.

DECLARATION OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

1 G. Varela-Moreiras, J. M. Ávila, C. Cuadrado, S. del Pozo, E. Ruiz and O. Moreiras, Eur. J. Clin. Nutr., 2010, 64, S37–S43.

2 P. A. Gilbert and S. Khokhar, Nutr. Rev., 2008, 66, 203–215.

3 ENIDE, 2011. Spanish National Food Safety and Nutrition Agency. http://aesan.msssi.gob.es/AESAN/docs/docs/evaluacion_riesgos/estudios_evaluacion_nutricional/valoracion_nutricional_enide_macronutrientes.pdf. (accessed November 2015).

4 S. Fiszman, in Advances in deep-frying of foods, ed. S. Sahin and S. G. Sumnu, CRC Press, Boca Raton, FL, 2009, ch. 11.

5 C. Delgado-Andrade, M. Mesías, F. J. Morales, I. Seiquer and M. P. Navarro, LWT – Food Sci. Technol., 2012, 6, 16–22.

6 E. K. Paleologos and M. G. Kontoninas, J. Food Prot., 2007, 70, 466–470.

7 T. Pérez-Palacios, C. Petisca, R. Henriques and I. M. P. L. V. O. Ferreira, Food Chem. Toxicol., 2013, 55, 222–228.

8 EFSA, 2015. EFSA J., 13, 4104. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/4104.pdf. (accessed November 2015).

9 J. E. Hodge, J. Agric. Food Chem., 1953, 1, 928–943.

10 L. W. Kroh, Food Chem., 1994, 51, 373–379.

11 H. Glatt, H. Schneider and Y. Liu, Mutat. Res., Genet. Toxicol. Environ. Mutagen., 2005, 580, 41–52.

12 A. H. Høie, C. Svendsen, G. Brunborg, H. Glatt, J. Alexander, W. Meinl and T. Husøy, Environ. Mol. Mutagen., 2015, 56, 709–714.

13 EFSA, EFSA J., 2005, 215, 1–73. http://www.efsa.europa.eu/en/scdocs/scdoc/215.htm. (accessed November 2015).

14 F. J. Morales and G. Arribas-Lorenzo, Food Chem., 2008, 109, 421–425.

15 AOAC, Official Method of Analysis of AOAC International, Association of Official Analytical Chemists, Maryland, 1999.

16 G. L. Miller, Anal. Chem., 1959, 31, 426–428.

17 C. Martínez-Villaluenga, P. Gulewicz, J. Frias, K. Gulewicz and C. Vidal-Valverde, Eur. Food Res. Technol., 2008, 226, 1465–1478.

18 V. Gökmen, A. Serpen and V. Fogliano, Trends Food Sci. Technol., 2009, 20, 278–288.

19 V. L. Singleton, R. Orthofer and R. M. Lamuela-Raventós, Methods Enzymol., 1999, 299, 152–178.

20 A. Horszwald, F. J. Morales, M. D. del Castillo and H. Zielinski, J. Food Nutr. Res., 2010, 49, 149–159.

21 J. A. Rufián-Henares, C. Delgado-Andrade and F. J. Morales, Food Chem., 2009, 114, 93–99.

22 M. Mesías, F. Holgado, G. Márquez-Ruiz and F. J. Morales, LWT – Food Sci. Technol., 2015, 62, 633–639.
23 E. Guerra-Hernández, in Acrylamide in Food. Analysis, content and potential health effects, ed. V. Gökmen, Academic Press, London, UK, 2015, ch. 13.
24 A. Claus, P. Schreiter, A. Weber, S. Graeff, W. Herrmann, W. Claupein, A. Schieber and R. Carle, J. Agric. Food Chem., 2006, 54, 8968–8976.
25 L. Ahrné, C. G. Andersson, P. Floberg, J. Rosén and H. Lingnert, LWT, 2007, 40, 1708–1715.
26 D. Huang, B. Ou and R. L. Prior, J. Agric. Food Chem., 2005, 53, 1841–1856.
27 A. Michalska, M. Amigo-Benavent, H. Zielinski and M. D. del Castillo, J. Cereal Sci., 2008, 48, 123–132.
28 S. Jensen, H. Oestdal, M. R. Clausen, M. L. Andersen and L. H. Skibsted, LWT – Food Sci. Technol., 2011, 44, 637–642.
29 D. Firestone, in Deep Frying: Chemistry, Nutrition and Practical Applications, ed. M. D. Erickson, AOCS Press, Champaign, 2007, ch. 21.
30 M. Friedman, J. Agric. Food Chem., 1996, 44, 631–653.
31 M. J. Antal, W. S. L. Mok and G. N. Richards, Carbohydr. Res., 1990, 199, 91–109.
32 C. Perez Locas and V. A. Yaylayan, J. Agric. Food Chem., 2008, 56, 6717–6723.
33 T. M. Amrein, B. Schönbächler, F. Escher and R. Amador, J. Agric. Food Chem., 2004, 52, 4282–4288.
34 Food and Drink Europe (FDE), 2013. http://www.fooddrinkeurope.eu/uploads/publications_documents/Acrylamide-Toolbox_2013.pdf. (accessed November 2015).
35 M. Ngadi, Y. Li and S. Oluka, Lebensm. – Wiss. Technol., 2007, 40, 1784–1791.
36 J. L. Quiles, M. C. Ramírez-Tolosa, J. A. Gómez, J. R. Huertas and J. Mataix, Food Chem., 2002, 46, 461–468.
37 I. Elisia and D. D. Kitts, CAB Rev., 2008, 3, 076.
38 M. L. I. Fei, L. I. Tong, L. I. Wei and L. De Yang, Ind. Crops Prod., 2015, 69, 137–142.
39 S. Žilić, T. Kocadağlı, J. Vančetović and V. Gökmen, LWT – Food Sci. Technol., 2016, 65, 597–603.
40 Ö. Ç. Açar and V. Gökmen, Mol. Nutr. Food Res., 2009, 53, 1521–1525.
41 Y. Miao, H. Zhang, L. Zhang, S. Wu, Y. Sun, Y. Shan and Y. Yuan, J. Food Sci. Technol., 2014, 51, 4005–4011.
42 N. Totani, M. Yawata, M. Takada and M. Moriya, J. Oleo Sci., 2007, 56, 103–106.

FIGURES AND TABLES

Table 1. Characterization of breadcrumb (BC) and fried breadcrumb samples (frying conditions 180°C, 3 min).

	BC-1	BC-2	BC-3	BC-4	BC-5	BC-6
Breadcrumbs						
Moisture (%)	7.2 ± 0.0cB	5.8 ± 0.1aB	7.4 ± 0.0dB	6.8 ± 0.0bB	8.2 ± 0.0eB	6.7 ± 0.1bB
Water activity	0.35 ± 0.00cB	0.28 ± 0.00aB	0.36 ± 0.00dB	0.32 ± 0.00bB	0.40 ± 0.00eB	0.35 ± 0.00cB
pH	7.8 ± 0.0e	6.3 ± 0.0c	6.1 ± 0.0a	6.7 ± 0.0d	6.2 ± 0.0b	6.2 ± 0.0b
Protein (g 100 g^{-1})	9.5 ± 0.0b	10.7 ± 0.1d	9.5 ± 0.1b	12.7 ± 0.0e	9.2 ± 0.0a	10.1 ± 0.0c
Reducing sugars (g 100 g^{-1})	2.1 ± 0.0a	6.7 ± 0.1e	6.6 ± 0.1e	3.0 ± 0.1b	5.5 ± 0.1d	5.1 ± 0.0c
Free Asn (mg 100 g^{-1})	14.3 ± 2.3b	3.2 ± 0.9a	12.5 ± 1.8b	16.4 ± 2.1b	13.1 ± 1.7b	25.3 ± 1.2c
E index	81.3 ± 0.6dB	77.4 ± 0.3bB	79.2 ± 0.9cB	81.6 ± 0.4dB	80.9 ± 0.7dB	73.4 ± 0.8aB
HMF (mg kg^{-1})	< LOQ	14.6 ± 0.7cA	19.4 ± 1.1dA	0.6 ± 0.0aA	2.6 ± 1.0bA	2.0 ± 0.5bA
Furfural (mg kg^{-1})	< LOQ	1.3 ± 0.3bA	1.4 ± 0.2bA	< LOQ	0.4 ± 0.1aA	0.5 ± 0.0aA
Acrylamide (µg kg^{-1})	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
ABTS (µmol TEAC g^{-1})	10.1 ± 0.3aA	22.1 ± 0.2cB	23.8 ± 0.3dB	20.3 ± 0.2bA	20.8 ± 0.1bB	22.6 ± 0.3cA
FCRC (GAE µg g^{-1})	33.1 ± 6.9bA	46.2 ± 1.4cA	23.8 ± 5.1aA	31.4 ± 3.0bA	20.9 ± 6.7aA	34.8 ± 4.0bA
Fried breadcrumbs						
Moisture (%)	2.2 ± 0.0bA	2.2 ± 0.0bA	2.1 ± 0.1bA	3.0 ± 0.0dA	1.6 ± 0.2aA	2.5 ± 0.0cA
Water activity	0.17 ± 0.01cA	0.12 ± 0.00abA	0.13 ± 0.01bA	0.26 ± 0.00dA	0.11 ± 0.00aA	0.16 ± 0.00cA
Oil uptake (%)	28.8 ± 0.1b	35.1 ± 0.0e	33.3 ± 0.1d	35.7 ± 0.0f	27.5 ± 0.1a	31.8 ± 0.1c
E index	56.3 ± 0.3cA	51.2 ± 0.7aA	52.7 ± 0.3abA	53.3 ± 0.4bA	57.6 ± 0.2dA	52.0 ± 1.2aA
HMF (mg kg^{-1})	7.8 ± 0.3a	98.5 ± 9.2fB	33.8 ± 0.9dB	29.2 ± 0.2cB	25.5 ± 0.4bB	47.2 ± 0.6eB
Furfural (mg kg^{-1})	4.1 ± 0.1a	10.4 ± 0.8dB	5.3 ± 0.1bcB	5.0 ± 0.0b	3.7 ± 0.1aB	5.9 ± 0.1cB
Acrylamide (µg kg^{-1})	538 ± 21d	78 ± 9a	262 ± 18b	525 ± 0d	250 ± 14b	414 ± 6c
ABTS (µmol TEAC g^{-1})	19.0 ± 0.2cB	16.5 ± 0.4bA	15.6 ± 0.3aA	22.2 ± 0.2dB	16.2 ± 0.5bA	22.3 ± 0.2dA
FCRC (GAE µg g^{-1})	221.5 ± 20.3bB	250.1 ± 9.9cB	206.1 ± 9.8abB	286.4 ± 8.5cdB	191.7 ± 5.1aB	299.4 ± 5.8dB

Asn: Asparagine. HMF: Hydroxymethylfurfural. ABTS: 2,2′-Azobis-(3-ethylbenzothiazoline-6-sulfonic acid). TEAC: Trolox equivalent antioxidant capacity. FCRC: Folin–Ciocalteau reducing capacity. GAE: Gallic acid equivalent. LOQ: Limit of quantification.

Different small letters mean significant differences among the six samples for a same parameter ($p < 0.05$). Different capital letters mean significant differences between non-fried and fried breadcrumbs for a same sample in the same parameter ($p < 0.05$).

Fig. 1. Bread coat model in stainless steel bags

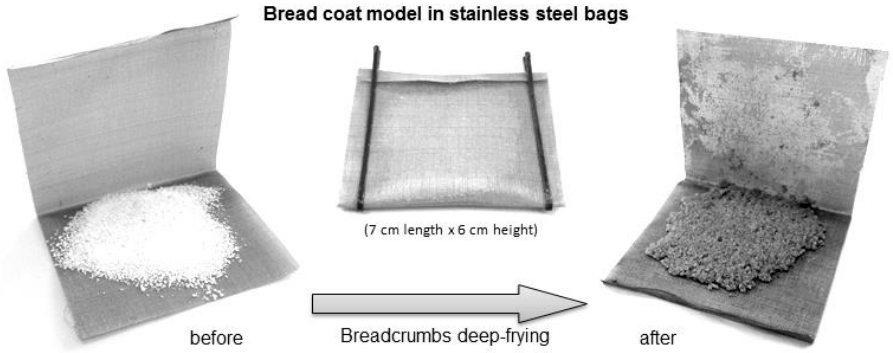

Fig. 2. Relationship between asparagine and reducing sugars (RS) content with acrylamide formation in breadcrumb (BC) samples fried at 180°C for 3 minutes

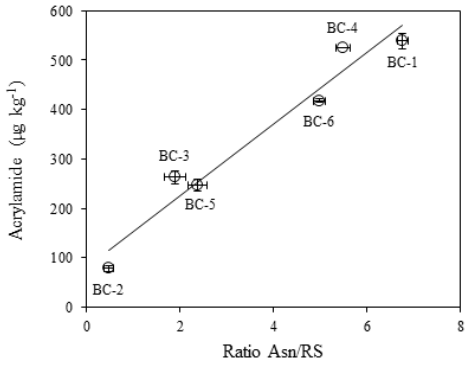

Fig. 3. HMF, furfural and acrylamide formation in breadcrumb (BC-1, BC-2 and BC-5) during deep frying at 180ºC for different times

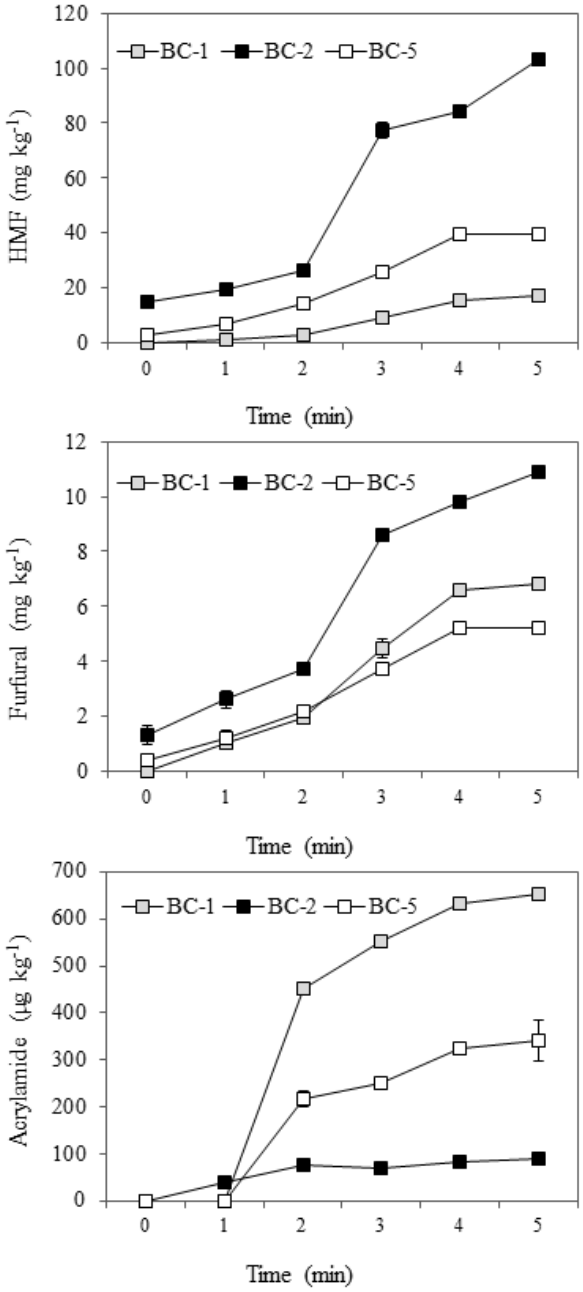

Fig. 4. HMF, furfural and acrylamide formation in breadcrumb (BC-1, BC-2 and BC-5) during deep frying for 3 minutes at different temperatures

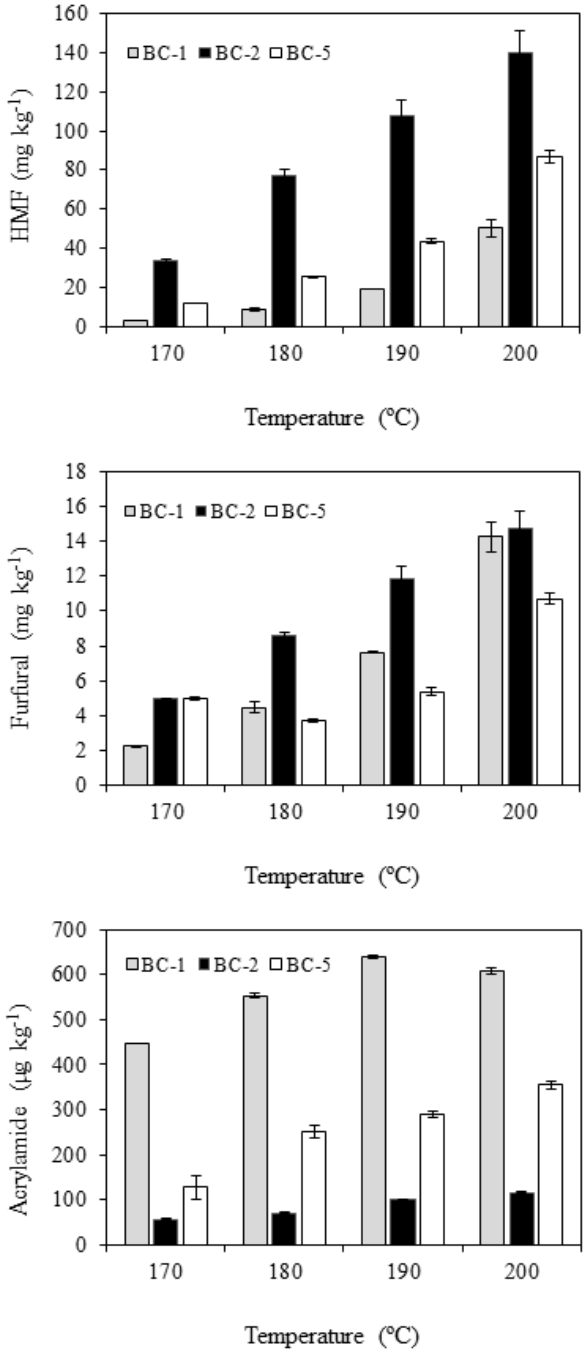

Fig. 5. Changes in the antioxidant capacity and Folin-Ciocalteau reducing capacity (FCRC) in breadcrumb (BC-1, BC-2 and BC-5) during deep frying at 180°C for different times

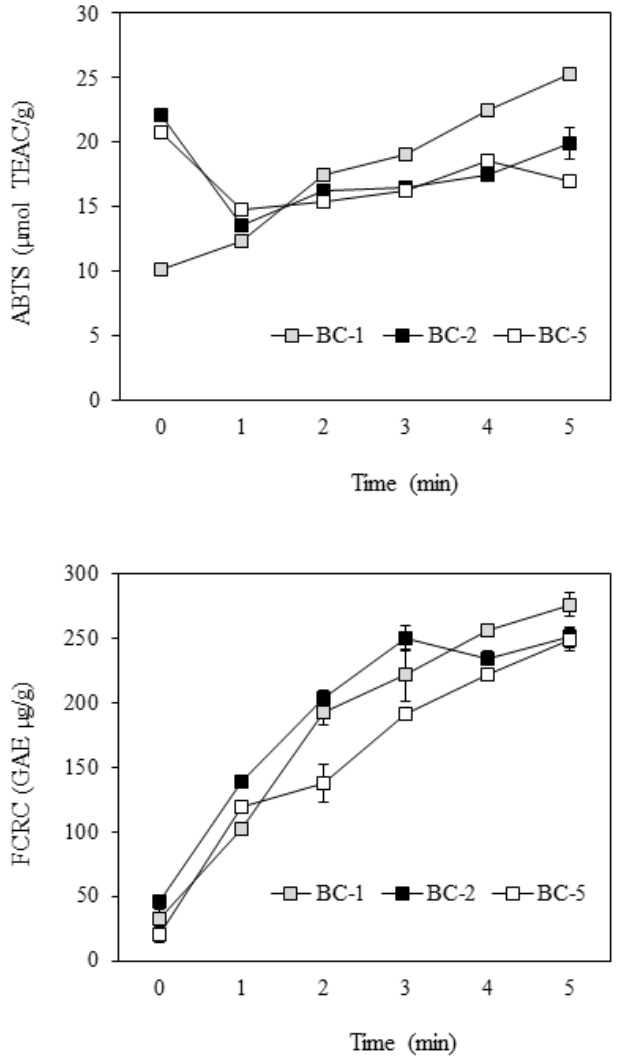

Fig. 6. Changes in the antioxidant capacity and Folin-Ciocalteau reducing capacity (FCRC) in breadcrumb (BC-1, BC-2 and BC-5) during deep frying for 3 minutes at different temperatures