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Relationship between acrylamide and thermal-processing indexes in commercial breakfast cereals: A survey of Spanish breakfast cereals

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Breakfast cereals are significant contributors to the daily intake of food-derived acrylamide in Western countries. Acrylamide was determined by LC-MS in 60 commercial breakfast cereals marketed in Spain. Several SPE cartridges were evaluated for clarification of the aqueous extract. LOQ was 62 μg/kg. Acrylamide content ranged from <62–803 μg/kg (average 292 μg/kg, and median 258 μg/kg, with an estimated acrylamide intake from breakfast cereals of 2.68 μg acrylamide/person/day. According to the German concept of minimization, a signal value 450 μg/kg was calculated. Relationships among acrylamide and some parameters of the studied samples such as type of cereal, its physical form (puffed and flaked) or certain ingredients in the formulation (proteins and dietary fibre content) were also investigated. Wheat-based cereals contained significantly higher levels of acrylamide, as did samples with higher fibre or protein content. In addition, puffed breakfast cereals also contained significantly higher levels of acrylamide. There was no significant correlation between acrylamide levels and contents of 5-hydroxymethylfurfural, furosine or cereal browning.

Keywords: Acrylamide / Breakfast cereals / Browning / Furosine / HMF

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1 Introduction

In 2002, heat-induced formation of the free monomer acrylamide (CH_2=CHCONH$_2$) was reported in a wide variety of fried and oven-cooked foods, most notably in potato chips and French fries, at levels of 224–3700 μg/kg [1]. The presence of acrylamide in cooked foods has raised public concern about food safety because the International Agency for Research on Cancer has classified acrylamide as 'probably carcinogenic to humans' on the basis of sufficient evidence for carcinogenicity in experimental animals and mechanistic considerations [2]. Later, a joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that current acrylamide levels in foods may imply a human-health concern, being cancer the most important potential adverse effect of acrylamide consumption together with its neurotoxic effects [3].

Mottram and Wedzicha [4] and Stadler *et al.* [5] showed how acrylamide could be formed during heat treatment as a result of the Maillard reaction, involving mostly asparagine and glucose. Two main mechanisms of acrylamide formation in foods have been proposed [6, 7]. However, acrylamide is also generated by direct decarboxylation from Schiff bases prior to the Amadori rearrangement, as well from carnosine, Strecker aldehydes or from pyrolysis of gluten [8–10]. Finally, acrolein and ammonia have also been identified as precursors of acrylamide from thermal degradation of triglycerides in lipid-rich foods [11]. Acrylamide chemistry, biochemistry, occurrence, metabolism and toxicology have been reviewed elsewhere [12].

Investigations have been focussed on food commodities with high acrylamide formation, such as potato-derived foodstuffs, or with significant impact on the dietary population habits, like cereal-based foodstuffs. Breakfast cereals comprise a huge family of products obtained by a range of different technological processes applied to a variety of cereal crops. Generally, manufacture of breakfast cereals involves extrusion (under intermediate a_w, and temperature over 80–95°C) and drying-toasting step (low a_w, and temperatures higher than 150°C), both of them favouring the main chemical reactions involved during breakfast cereals manufacture: Maillard reaction and caramelization.

Traditionally, measurement of colour, hydroxymethylfurfural (HMF) and furosine (ε-*N*-2-furoylmethyl-L-lysine) have been applied as chemical indexes of thermal proces-

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Abbreviations: DEEC, direct expansion extrusion cooking; **HMF,** hydroxymethylfurfural

sing in different cereal products such as baby and breakfast cereals [13 – 15), pasta [16] and bakery products [17, 18]. Analytical methodologies for analysis of HMF, furosine and cereal browning are well established. However, acrylamide is an emerging harmful substance from Maillard reaction in such products where use of more sophisticated laboratory equipment is required.

Therefore, one of the purposes of the present investigation was to study the relationship between acrylamide levels with some heat-induced parameters such as HMF, furosine and browning in order to assess their usefulness for predicting the potential acrylamide levels in commercial breakfast cereals. In addition, the acrylamide content of commercial breakfast cereals marketed in Spain as a key source of acrylamide into the diet was carried out in a survey. Finally, the relationship among levels of acrylamide and compositional parameters of the samples (*i. e.* type of cereal, protein content and addition of dietary fibre) or manufacturing processes (flaking or puffing) were investigated.

2 Materials and methods

2.1 Samples

Experiments were conducted with a series of commercial breakfast cereals randomly purchased from different supermarkets (60 samples). Samples (300 – 500 g) were mixed and thinly grinded to assure a homogeneous distribution. A portion of powdered sample (200 g) was distributed in four containers and stored under vacuum and protected from light at 4°C under 2 months prior to analysis.

2.2 Chemicals and materials

[$^{13}C_3$]-acrylamide (isotopic purity 99%) was from Cambridge Isotope Labs (Andover, MA, USA). Acrylamide (99%), potassium ferrocyanide (Carrez I) and zinc acetate (Carrez II) were from Sigma-Aldrich (St.-Louis, MO, USA). Acetic acid (ultrapure grade) and Pronase E were from Merck (Darmstadt, Germany). Methanol and ACN (HPLC grade) were from Scharlau (Barcelona, Spain). The SPE cartridges Isolute® Multimode (500 mg, 3 mL) were from IST (Hewgoed, Mid-Glamorgan, UK); the RP Oasis® HLB (200 mg, 6 mL) and mixed-mode cation exchange cartridge Oasis MCX (60 mg, 3 mL) were from Waters (Milford, MA, USA); Xtrata® XC (60 mg, 3 mL and 200 mg, 6 mL) was from Phenomenex (Torrance, CA, USA); and Bond Elut® C_{18} (100 mg, 6 mL and 200 mg, 6 mL) from Varian (Palo Alto, CA, USA).

2.3 Acrylamide standard and reagents

Stock solutions of acrylamide (0.01 mg/mL) and [$^{13}C_3$]-acrylamide (5 μg/mL) were prepared by dissolving the compounds in Milli-Q water and methanol, respectively. These solutions were then conveniently diluted with Milli-Q water (Millipore, Madrid, Spain) to prepare working standards at 1.0 μg/mL. All stock solutions and working standards were stored light-protected in a refrigerator at 4°C up to 3 months. New working standards were compared with the previous one as a control for quality. Carrez I solution was prepared by dissolving 15 g of potassium ferrocyanide in 100 mL of water and Carrez II solution by dissolving 30 g of zinc acetate in 100 mL of water.

2.4 Sample extraction

Sample powder (0.75 g) was weighed with a precision of 0.1 mg and suspended in 8 mL of Milli-Q water in polypropylene centrifuge tubes. The suspension was spiked with 200 μL of a 5 μg/mL [$^{13}C_3$]-acrylamide methanolic solution as internal standard and mixed. Acrylamide extraction was performed at room temperature for 20 min, and shaking for 10 s every 10 min. In order to clarify the solution, 0.5 mL of each Carrez I and Carrez II solutions were added and finally the mixture was centrifuged (9000 $\times g$ *per* 10 min *per* 4°C).

2.5 Sample clean-up

Isolute Multimode SPE cartridges were preconditioned with 2 mL of methanol, 2 mL of water and 2 mL of air to remove the excess of water. An aliquot (1 mL) of the clear supernatant obtained above was loaded onto the cartridge at a flow rate of 2 mL/min. Then 2 mL of air was passed and finally acrylamide was eluted with 1 mL of water at the same flow rate. The solution was filtered through a 0.45 μm filter into an amber LC-MS vial.

2.6 LC-MS analysis

Sample extracts and calibration standards were analysed on an Agilent 1100 liquid chromatograph coupled to an Agilent Quadrupole MS detector (Agilent Technologies, Palo Alto, CA, USA). Analytical separation was achieved with a Luna ODS2 column (25 × 0.46 cm, 5 μm; Phenomenex) at 32°C. Isocratic elution was achieved with a mobile phase of acetic acid-methanol-Milli-Q water (0.1 : 2.5 : 97.4) at a flow rate of 0.8 mL/min. The injection volume was 80 μL. ESI in the positive ionization mode was used. The MS detector was operated in the SIM mode at *m/z* ratios of 72.1 and 75.1 for acrylamide and [$^{13}C_3$]-acrylamide, respec-

tively. Under these chromatographic conditions acrylamide eluted at 6.8 min. The needle and cone voltages were set at 3.0 kV and 100 V, respectively. Nitrogen was used as nebulizer gas (12.0 L/h) and the source temperature was set at 300°C.

2.7 Quantitation

Acrylamide was quantified using a linear calibration function that was established with standard solutions of acrylamide and [$^{13}C_3$]-acrylamide dissolved in Milli-Q water (25–1000 μg/L). Acrylamide contents in sample extracts were calculated from the calibration slope and intercept value, taking into account the recovery calculated by means of [$^{13}C_3$]-acrylamide slope. LOQ was 62 μg/kg on the basis of an S/N ratio of 10:1. Intraday repeatability of working reference material was 3.8%.

2.8 HMF determination

HMF determination was carried out by LC according to Rufian-Henares *et al.* [14].

2.9 Furosine determination

Furosine was determined according to Delgado-Andrade *et al.* [15].

2.10 Measurement of free and total coloured compounds (browning)

Absorbance from free coloured compounds was determined at 420 nm in the filtered extracts previously obtained for HMF measurement after adequate dilution. In the same way, absorbance from total coloured compounds (including those from free compounds and compounds linked to the protein backbone) was measured at the same wavelength in the diluted extracts obtained after enzymatic hydrolysis of samples. Briefly, 100 mg of sample was digested with 3 mL of a 0.375 mg/mL Pronase E solution (1500 U/mL in 1 M sodium-borate solution, pH 8.2) in a stoppered test tube for 36 h at 40°C in a water bath under shaking. After cooling, the solution was centrifuged at 4500 × *g* for 10 min at 4°C. The supernatant was filtered (0.45 μm) and adequately diluted for absorbance measurement. A Shimadzu UV-visible 1601 spectrophotometer (Duisburg, Germany) equipped with a UVPC personal spectroscopy software (v. 3. 9, Shimadzu Scientific Instruments, Germany) was used to perform both analyses. Analysis of colour was limited to samples with no added cocoa.

2.11 Statistical analysis

Statistical significance of data was tested by one-way analysis of the variance (ANOVA), followed by Duncan Test to compare means that showed significant variation ($P < 0.05$). Analyses were performed using Statgraphics Plus (version 5.1, 2001). At least, two independent analyses were carried out *per* sample.

3 Results and discussion

3.1 Method performance

3.1.1 Sample extraction

Extraction of free acrylamide monomer from the food matrix is a critical step since acrylamide may be firmly enclosed within the food matrix and not homogeneously distributed; in this sense standardized analytical procedure should be assayed in every different foodstuff in order to evaluate and reduce as much as possible the matrix interference [19]. Extraction was carried out for 30 min at room temperature (20–22°C) and at 60°C with a working reference breakfast cereal spiked with acrylamide. As also observed by some authors [20, 21], significant losses in acrylamide were recorded after 15 min of heating at 60°C (Fig. 1). Extraction in water for 20 min at room temperature revealed to be appropriate for acrylamide extraction in breakfast cereals. Furthermore, to evaluate the efficiency of the extraction, different amounts of sample were extracted with the same volume of water (data not shown). The highest recoveries were reached for 0.09 g of sample/mL. An increase of sample amount resulted in an excessively stiff paste (from 0.125 to 0.805 g of breakfast cereal), suggesting lower acrylamide recoveries.

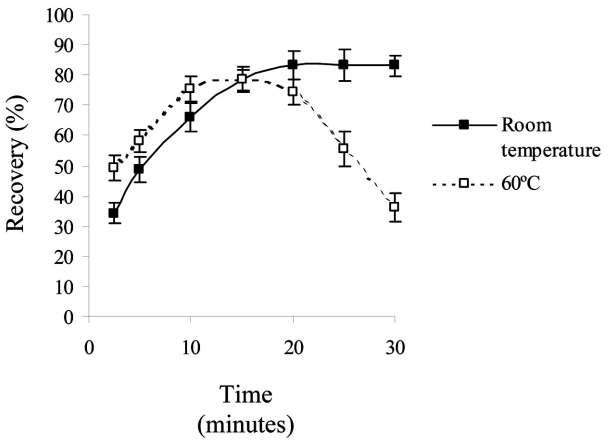

Figure 1. Effect of time and temperature on acrylamide recovery during aqueous extraction of breakfast cereals.

3.1.2 Sample clean-up

It is recommended to use some procedure for sample clean-up in order to remove coextractives and to enhance the detection limit by reducing ion suppression effects mainly for single quadrupole detection. The use of SPE cartridges is widely extended as compared with accelerated solvent extraction approaches, and to date several SPE cartridges have been refered in the literature, such as Isolute Multimode, OasisHLB, MCX, Bond Elut and Xtrata®. The characteristic features of the Isolute-Multimode sorbent are hydrophobic interaction (presence of C_{18} functional groups), and strong cationic (SCX) as well as anionic (SAX) exchange. On the other hand, HLB is a C_{18} cartridge, while MCX is an SCX. In the case of Xtrata-XC, it shows both hydrophobic interaction and SCX exchange and Bond Elut only hydrophobic characteristics. We tested all six cartridges using a working reference breakfast cereal with an acrylamide content of 428 ± 12 μg/kg. All the volume eluted from 1 mL of sample loaded was collected and analysed, although some dilution was expected from the first drops. Acrylamide is a highly polar compound and is poorly retained in traditional RP cartridges. As shown in Table 1, Isolute-Multimode achieved the highest recovery. Finally, the volume (0.8 – 1.2 mL) of sample loaded and elution flow rate were assayed (data not shown), selecting 1 mL for sample loading and a flow rate of 2 mL/min for acrylamide elution.

Table 1. Recovery of acrylamide by applying different commercial cartridges for SPE extraction ($n = 3$)

Cartridge	Recovery (%)
Isolute Multimode (500 mg)	81.7 ± 1.2
Oasis HLB + MCX (200 mg + 60 mg)	69.8 ± 0.9
Xtrata XC (60 mg)	67.2 ± 1.8
Bond Elut (100 mg)	62.2 ± 2.4
Bond Elut (200 mg)	52.5 ± 1.2
Xtrata XC (200 mg)	35.1 ± 0.8

3.2 Sample analysis

Sixty commercial breakfast cereals from 12 producers were analysed for acrylamide content. Acrylamide ranged from <62 to 803 μg/kg with an average value of 292 μg/kg and a median of 258 μg/kg (Table 2). Acrylamide contents lower than the LOQ were detected in five samples. Similar values were reported by other authors [22, 23] who found acrylamide levels ranging from <30 to 1346 μg/kg. Results are in line with those recently summarized in Spanish breakfast cereals [24, 25]. According to the most recent information, the consumption of breakfast cereals in Spain is 3.25 kg/person/year [26]. It has been estimated that the mean acrylamide intake from these products is around 2.68 μg acrylamide/person/day. This represents a high fraction of the daily intake of food-derived acrylamide and is of great importance since breakfast cereals consumption is steadily increasing in Spain (a 6% increase over the last year).

Table 2. Statistical treatment for acrylamide levels in breakfast cereals grouped according to the type of cereal, physical form, consumers, fibre content and protein content

Factor	Acrylamide (μg/kg)	No. of samples
Total		
Mean ± SD	292 ± 125	60
Median	258	
Minimum	<62	
Maximum	803	
Type of cereal		
Corn	207 ± 55[a)]	16
Rice	144 ± 34[a, b)]	21
Mixture	307 ± 108[a, c)]	3
Wheat	382 ± 136[d)]	20
Physical form		
Flaked	255 ± 73[a)]	21
Puffed	325 ± 143[b)]	39
Consumers		
Adult population	372 ± 166[a)]	5
Infant population	277 ± 103[b)]	45
Dietary fibre content		
>5%	401 ± 161[a)]	13
<5%	273 ± 102[b)]	47
Protein content		
>7.5%	362 ± 142[a)]	27
<7.5%	250 ± 87[b)]	33

Values represent mean ±SD.

[a – d)] Different letters within the same factor indicate statistical differences (One-way ANOVA and Duncan test, $p < 0.05$).

A more detailed study of acrylamide distribution in the studied samples was carried out, and a box-and-whisker plot was used since this graphical presentation uses a nonparametric test (Fig. 2). Many studies have been accomplished to find strategies to minimize the levels of acrylamide. This objective can be achieved either by modifying processing parameters such as pH or temperature/time of heating, or by acting on precursors or key intermediates. WHO and the Scientific Committee for Food of the European Union called for strategies to reduce acrylamide formation to a minimum by implementing the ALARA principle (as low as reasonably or technically achievable). The German Federal Office of Consumer Protection and Food Safety (BVL) stated a signal value 200 μg/kg for breakfast cereals, as a concept of minimization. Signal value is defined as the lowest level of the 10% containing the highest level of acrylamide. If acrylamide contents were above this signal value, food producers should be recommended to take adequate actions to lower such contents. When we applied the concept to our surveyed breakfast samples, most of the samples (87%) contained acrylamide levels higher than 200 μg/kg. Actually, a signal value of 450 μg/kg was established for breakfast cer-

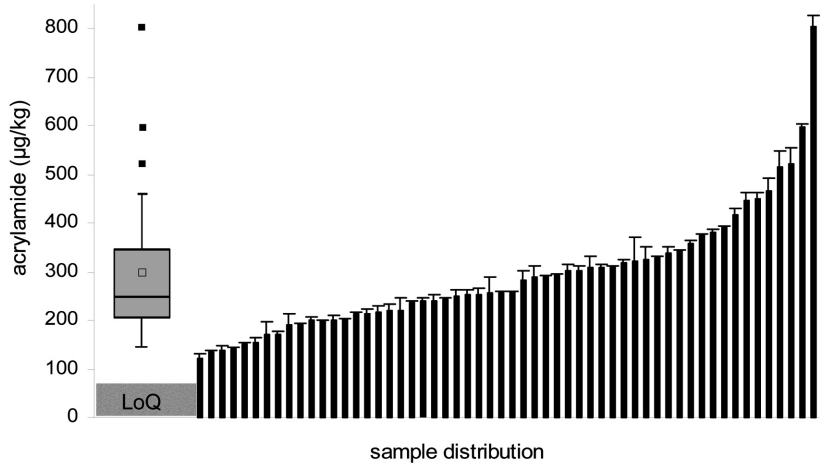

Figure 2. Bar graph and box-and-whisker plot of acrylamide content in commercial breakfast cereals. Five samples were under LOQ (62 µg/kg).

eals marketed in Spain. This is an important issue taking into account that the California Environmental Protection Agency (Office of Environmental Health Hazard Assessment) has proposed recently an alternative cancer risk level calculation, when consuming bread and cereals with an acrylamide concentration lower than 200 µg/kg (www.oehha.org/prop65/law/pdf_zip/Notice-%2012705eAltRisk revised.pdf).

As described previously, breakfast cereals comprise a heterogeneous group of food commodities. Samples were classified into four groups based on the type of flour used in the formulation (Table 2): corn ($n = 16$), wheat ($n = 21$), rice ($n = 3$) and cereal-mixture ($n = 20$) based products (the later mostly being mixtures with high proportions of wheat flour). Acrylamide content was significantly higher in wheat-based breakfast cereals. These results are in agreement with those previously reported [27] which showed a higher potential in acrylamide formation in wheat-based cereals followed by corn, oat and rice. Differences could be directly attributed to the protein content in the formulation, which mainly depends on the type of flour used. As described in the literature (CIAA, Confederation of the Food and Drink Industries of the UE (2005) The CIAA Acrylamide "Toolbox", rev. 6, September, CIAA, Brussels), asparagine is the main parameter that conditions acrylamide formation in cereal-derived products, whereas reducing sugars did not exert a significant effect in this group of foods. Moreover, addition of asparaginase causes a large reduction on acrylamide levels, while no effect of glucose oxidase was found [28].

Breakfast cereals usually have dietary fibre contents of about 3–4%, but when enriched with wheat-bran, dietary fibre increases up to 10–30%. As illustrated in Table 2, there were statistically significant differences in the acrylamide content between the dietary fibre added cereals (401 ± 161; $n = 13$) and those with no added dietary fibre (273 ± 102; $n = 47$). This could be explained by the fact that asparagine, the main precursor of acrylamide [6] in cereal products, is concentrated in the bran, particularly in wheat-bran (CIAA, Confederation of the Food and Drink Industries of the UE (2005) The CIAA Acrylamide "Toolbox", rev. 6, September, CIAA, Brussels). In this sense, breakfast cereals destined to the adult population contained higher acrylamide levels than those for children (Table 2). This effect could be explained taking into account that commercial breakfast cereals focussed to adult consumers had higher protein content (11.86%) than those for the infant population (6.66%); on the other hand, the former cereals are mostly enriched in dietary fibre (mainly wheat-bran) because of the healthy orientation of this kind of products.

Finally, differences in acrylamide were also linked to the industrial processing technology applied. This aspect could not be directly evaluated since sampling was carried out at the supermarkets and no information was supplied by manufacturers. In contrast to potato food commodities, there is still a lack of information on facts affecting acrylamide formation in cereals or bakery wares [29]. Acrylamide formation not only depends on its precursors, asparagine and reducing sugars, but also on enzymatic degradation of starch and proteins during dough preparation or further treatments. Breakfast cereals, when presented as flakes, are usually manufactured by the extrusion process called PFEC (Pellet-to-flaking extrusion cooking), but other types of breakfast cereals (*i.e.* puffed cereals) are usually manufactured by direct expansion extrusion cooking (DEEC process) [30]. Thus, because of the higher moisture content of puffed breakfast cereals, a strong drying and tempering step must be performed in order to equilibrate humidity to approximately 10% to improve shelf stability [31]. Higher thermal input during the DEEC process could give rise to acrylamide values higher than those observed in breakfast cereals manufactured by the PFEC process. The former technological description fits with the results obtained from the comparison between breakfast cereals in the flaked and puffed forms. Statistically significant differences were

found between flakes and puffed products, with higher acrylamide levels in puffed breakfast cereals (Table 2).

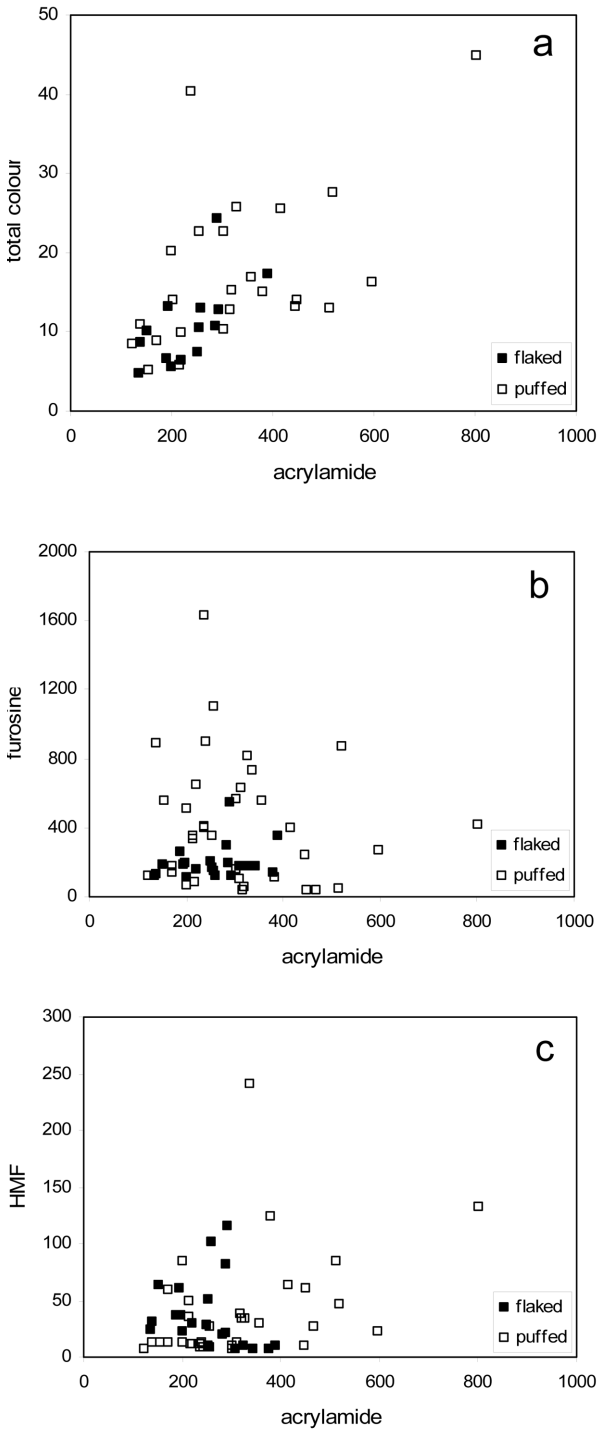

Figure 3. Relationships between a) colour (absorbance at 420 nm), b) furosine (mg/100 g protein), and c) hydroxymethylfurfural (mg/kg product) with acrylamide (μg/kg product) in commercial breakfast cereals. Flaked form (solid box), puffed form (empty box) breakfast cereals.

3.3 Relationship between acrylamide and browning indicators

HMF, furosine and colour development are classical indicators of the thermal treatment applied to breakfast cereals. HMF, furosine and colour (free and linked-to-proteins absorbance at 420 nm) were analysed and correlated to the acrylamide content in order to determine the possible direct relationships between acrylamide and these browning indicators. It is important to carry out the study in real commercial samples instead of industrial- or pilot-scaled samples since both ingredients and processing conditions are not sufficiently well-controlled. There was no statistically significant correlation between HMF, furosine or colour with acrylamide levels in commercial breakfast cereals (Fig. 3a–c). This lack of correlation with colour did not agree with results reported by other authors for fried potato-derived foods, bread crust, rice crisp bread, wheat flour and coffee [29, 32–35] since studies were placed under controlled conditions and the same raw material was used. In model systems, Ehling and Shibamoto [36] showed a clear correlation between formation of acrylamide and browning colour as well with formation of pyrazines. In general, a relative correlation between browning and acrylamide content exists in small surface products but not in large surface products [37], and become significant when ingredients and thermal-processing conditions are under control.

4 Concluding remarks

An average acrylamide content of 292 μg/kg ($n = 60$) in commercial breakfast cereals marketed in Spain is reported. Thus, dietary intake of acrylamide for the Spanish population for risk assessment purposes can be estimated, showing an estimated intake of 2.68 μg acrylamide/person/day.

In addition, this study illustrates the relationship among levels of acrylamide and compositional parameters such as the type of cereal, protein content and dietary fibre content. The type of cereal used in the formulation reveals to be critical for the acrylamide levels, showing that wheat-based cereals had the highest acrylamide contents. Commercial breakfast cereals enriched in dietary fibre, which are associated to healthy dietary habits and specially aimed to the adult population, showed the highest acrylamide contents. Finally, the DEEC process for breakfast cereals production in the puffed form has a direct impact on the generation of acrylamide, because of the more severe thermal treatment applied.

No significant relationships between acrylamide contents and HMF, furosine or coloured compounds (browning at 420 nm) were found. Therefore, these classical browning indicators will not be useful to the Administration for legis-

lative purposes in order to define limits of actuation since they are not directly related to the acrylamide content in commercial breakfast cereals. However, it could be feasible that relationships between these parameters and acrylamide formation become significant at pilot- or industrial-scales under controlled conditions (formulation) or at the same processing line.

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5 References

[1] Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Tornqvist, M., *J. Agric. Food Chem.* 2002, *50*, 4998 – 5006.

[2] IARC (1994, 08/26/1997), *Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Industrial Chemicals*, Lyon, IARC, Vol. 60.

[3] FAO/WHO. *Summary and Conclusions of the 64th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*, Geneva: FAO/WHO 2005.

[4] Mottram, D. S., Wedzicha, B. L., *Nature* 2002, *419*, 448 – 449.

[5] Stadler, R. H., Blank, I., Varga, N., Robert, F., *et al.*, *Nature* 2002, *419*, 449 – 450.

[6] Becalski, A., Lau, B. P., Lewis, D., Seaman, S. W., *J. Agric. Food Chem.* 2003, *51*, 802 – 808.

[7] Zyzak, D. V., Sanders, R. A., Stojanovic, M., Tallmadge, D. H., *et al.*, *J. Agric. Food Chem.* 2003, *51*, 4782 – 4787.

[8] Yaylayan, V. A., Wronowski, A., Peres, L. C., *J. Agric. Food Chem*. 2003, *51*, 1753 – 1757.

[9] Yaylayan, V. A., Perez, L. C., Wnorowski, A., O`Brien, J., *J. Agric. Food Chem*. 2004, *52*, 5559 – 5565.

[10] Claus, A., Weisz, G. M., Schieber, A., Carle, R., *Mol. Nutr. Food Res.* 2006, *50*, 87 – 93.

[11] Yasuhara, A., Tanaka, Y., Hengel, M., Shibamoto, T., *J. Agric. Food Chem*. 2003, *51*, 3999 – 4003.

[12] Friedman, M., *J. Agric. Food Chem*. 2003, *51*, 4504 – 4526.

[13] Guerra-Hernández, E., Corzo, N., *Cereal Chem.* 1996, *73*, 729 – 731.

[14] Rufian-Henares, J. A., Delgado-Andrade, C., Morales, F. J., *J. AOAC Int*. 2006, *89*, 161 – 165.

[15] Delgado-Andrade, C., Rufian-Henares, J. A., Morales, F. J., *Eur. Food Res. Technol.* 2005, *221*, 707 – 711.

[16] Resmini, P., Pellegrino, L., Pagani, M. A., De Noni, I., *Ital. J. Food Sci.* 1993, *4*, 341 – 353.

[17] Ramírez-Jiménez, A., García-Villanova, B., Guerra-Hernández, E., *Food Res. Int.* 2000, *33*, 833 – 838.

[18] Rada-Mendoza, M., Sanz, M. L., Olano, A., Villamiel, M., *Food Chem*. 2004, *85*, 605 – 609.

[19] Biedermann, M., Biedermann-Brem, S., Noti, A., Grob, B., *Mit. Lebensm. Hyg.* 2002, *93*, 638 – 652.

[20] Castle, L., *J. Agric. Food Chem.* 2003, *41*, 1261 – 1263.

[21] Rosén, J., Hellenäs, K. E., *Analyst* 2002, *127*, 880 – 882.

[22] Riediker, S., Stadler, R. H., *J. Chromatogr. A* 2003, *1020*, 121 – 130.

[23] Roach, J. A. G., Andrzejewski, D., Gay, M. L., Nortrup, D., Musser, M., *J. Agric. Food Chem.* 2003, *51*, 7547 – 7554.

[24] Yusa, V., Quintas, G., Pardo, O., Marti, P., Pastor, A., *Food Addit. Contam.* 2006, *23*, 237 – 244.

[25] Bermudo, E., Motano, E., Pignou, L., Galceran, M. T., *Anal. Chim. Acta* 2006, *5*, 207 – 214.

[26] MAPA (2003), *La alimentación en España* Ministerio de Agricultura, Pesca y Alimentación, Ed., Madrid, Spain 2003.

[27] RHM Technologies, *Presentation at the FSA Process Contaminants Meeting*, April 2005, London, UK.

[28] Knutsen, S. H., Brathen, E., Syversen, J. T., *Næringsmiddelindustrien* 2003, *5*, 10 – 12.

[29] Surdyk, N., Rosen, J., Andersson, R., Aman, P., *J. Agric. Food Chem*. 2004, *52*, 2047 – 2051.

[30] Camire, M. E., Camire, A., Krumbar, K., *Crit. Rev. Food Sci. Nutr.* 1990, *29*, 35 – 57.

[31] Eastman, J., Orthoefer, F., Solorio, S., *Cereal Foods World* 2000, *46*, 468 – 471.

[32] Pedreschi, F., Kaack, K., Granby, K., *Food Res. Inter.* 2006, *39*, 40 – 46.

[33] Gökmen, V., Senyuva, H. Z., *Food Chem*. 2006, *99*, 238 – 243.

[34] Olsson, K., Svensson, R., Roslund, C. A., *J. Sci. Food Agric.* 2004, *84*, 447 – 458.

[35] Mustafa, A., Andersson, R., Rosén, J., Kamal-Eldin, A., Aman, P., *J. Agric. Food Chem*. 2005, *53*, 5985 – 5989.

[36] Ehling, S., Shibamoto, T., *J. Agric. Food Chem*. 2005, *53*, 4813 – 4819.

[37] Taubert, D., Harlfinger, S., Henkes, L., Berkels, R., Schomig, E., *J. Agric. Food Chem*. 2004, *52*, 2735 – 2739.

