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## Acrylamide content of selected Spanish foods: A survey of biscuits and bread derivatives

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# 1 Acrylamide content of selected Spanish foods: A survey of biscuits and 2 bread derivatives

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## 16 17 18 19 20 21 **Abstract**

22 An overview of the acrylamide content in commercial biscuits and bread derivatives  
23 (bread sticks, bread crust, crackers) marketed in Spain is presented. Acrylamide was  
24 determined by stable isotope dilution LC-MS with an LOQ of 30  $\mu\text{g kg}^{-1}$ . Acrylamide  
25 content ranged from <LOQ -2085  $\mu\text{g kg}^{-1}$ , <LOQ -151  $\mu\text{g kg}^{-1}$ , <LOQ - 296  $\mu\text{g kg}^{-1}$ ,  
26 <LOQ - 23  $\mu\text{g kg}^{-1}$  for biscuits, crisp bread, crackers and bread sticks. Acrylamide was  
27 significantly higher in samples when ammonium hydrogen carbonate had been used as a  
28 rising agent and high fibre content (> 5%) used in the formulation, but lower when  
29 functional ingredients such as polyols were used. An estimation of the acrylamide  
30 dietary exposure related to biscuits and bread-derivatives was calculated as 0.082  $\mu\text{g kg}^{-1}$   
31  $\text{day}^{-1}$ . Estimated dietary intake ranged from 0.002 to 0.058  $\mu\text{g kg}^{-1} \text{day}^{-1}$  for crackers  
32 and biscuits respectively.

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51 **Keywords:** *Acrylamide, biscuits, crisp bread, crackers, bread sticks, food*  
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54 *contaminants.*

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## 2 **Introduction**

3 The detection of acrylamide, which has been classified as a probable carcinogen by the  
4 International Agency for Research on Cancer (IRAC, 1994), in a large variety of heated  
5 foods (Tareke et al. 2002) has created much concern among regulating authorities, the  
6 food industry and the public. Consequently, worldwide monitoring of acrylamide in  
7 different food types was started (Lineback et al. 2005). Extensive data on levels of  
8 acrylamide have been collected by the European Commission (2005) and by the US  
9 Food and Drug Administration (2004). Among the most important matrices included in  
10 the European monitoring database are potato chips, crispbread, breakfast cereals and  
11 biscuits.

12  
13 Acrylamide is formed in the Maillard reaction, reducing sugars such as glucose and  
14 fructose, and the amino acid asparagines being the major reactants (Stadler et al. 2002,  
15 2004; Zyzak et al. 2003). Two mechanisms, varying in details, have been proposed  
16 (Becalski et al. 2003; Zyzak et al. 2003). However, evidence clearly points to the  
17 reaction between asparagine and reducing sugars as the main culprit.

18  
19 It is now well-established that processing conditions such as time, temperature, and  
20 matrix influence acrylamide formation and degradation (Friedman, 2003). Acrylamide  
21 formation was found to occur during the browning process at temperatures above 120  
22 °C (Mottram et al. 2002; Yaylayan et al. 2003). In the case of starchy products (mainly  
23 bakery) acrylamide contents up to 2000 µg/kg were observed (Croft et al. 2004). The  
24 highest contents were often found in products, such as gingerbread, prepared with the  
25 baking agent ammonium hydrogen carbonate (Amrein et al. 2004; Konings et al. 2003)

1 which has been shown that strongly promotes the acrylamide formation in sweet bakery  
2 (Biedermann et al. 2003; Graf et al. 2006).

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10 As stated above investigations about acrylamide formation has been focused on food  
11 commodities with high acrylamide formation, such as potato-derived foodstuffs, or with  
12 significant impact on the dietary population habits, like cereal-based foodstuffs. Biscuits  
13 and bread-derivatives comprise a huge family of products obtained by baking a variety  
14 of cereal crops. Generally, manufacture of both kinds of products involves different  
15 steps such as mixture of ingredients and kneading, dough fermentation (mainly in bread  
16 crust and bread sticks), baking -up to 250°C for 15-30 min and in some cases a final  
17 toasting step (Ramirez-Jimenez et al. 2000). This last step favour the main chemical  
18 reactions involved during biscuits or bread derivatives manufacture, the Maillard  
19 reaction and caramelisation, which are responsible for the colour and flavour of these  
20 kinds of products (Ramirez-Jimenez et al. 2000).

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39 The study presented here should give an overview of the acrylamide content of  
40 commercial biscuits and bread derivatives marketed in Spain. These products, because  
41 of their relatively widespread consumption, could be a key source of acrylamide in the  
42 Spanish diet. Therefore, the relationship between acrylamide intake and population  
43 factors is also presented. In addition the correlation among levels of acrylamide and  
44 compositional parameters of the samples (i.e. presence of ammonium hydrogen  
45 carbonate, dietary fibre content and biscuit speciality) was investigated.

## 46 47 48 49 50 51 52 53 54 55 56 57 58 **Materials and methods**

### 59 60 *Samples*

1 Experiments were conducted with a series of commercial biscuits (62 samples), bread  
2 crust (24 samples), bread sticks (10 samples) and crackers (11 samples) randomly  
3 purchased on different supermarkets from the Autonomous Community of Madrid  
4 (Spain). The distribution of samples was representative of the proportions of these food  
5 commodities in the Spanish market. Samples (300-500 g) were mixed and finely ground  
6 to ensure homogeneous distribution. Sub-samples (200 g) were divided into 4  
7 containers and stored under vacuum and light protected at 4°C until analysis.

### 8 9 *Chemicals and materials*

10 [<sup>13</sup>C<sub>3</sub>]-acrylamide (isotopic purity 99%) was from Cambridge Isotope Labs (Andover,  
11 MA, USA). Acrylamide (99%), potassium ferrocyanide (Carrez I), zinc acetate (Carrez  
12 II) were from Sigma-Aldrich (St-Louis, MO, USA). Acetic acid (ultrapure grade) and  
13 Pronase E were from Merck (Darmstadt, Germany). Methanol and acetonitrile (HPLC  
14 grade) were from Scharlau (Barcelona, Spain). The solid-phase extraction (SPE)  
15 cartridges Isolute<sup>®</sup> Multimode (500 mg, 3 ml) were from IST (Hewgoed, Mid-  
16 Glamorgan, UK)

### 17 18 *Acrylamide standard and reagents*

19 Stock solutions of acrylamide (0.01 mg ml<sup>-1</sup>) and [<sup>13</sup>C<sub>3</sub>]-acrylamide (5 µg ml<sup>-1</sup>) were  
20 prepared by dissolving the compounds in Milli-Q water and methanol respectively.  
21 These solutions were then appropriately diluted with Milli-Q water (Millipore Corp.,  
22 Madrid, Spain) to prepare working standards at 1.0 µg ml<sup>-1</sup>. All stock solutions and  
23 working standards were stored light-protected in a refrigerator at 4°C up to 3 months.  
24 Carrez I solution was prepared by dissolving 15 g of potassium ferrocyanide in 100 ml  
25 of water and Carrez II solution by dissolving 30 g of zinc acetate in 100 ml of water.

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### *Acrylamide analysis*

Acrylamide was analysed as described by Rufian-Henares et al. (2006) with some minor modifications.

*Sample extraction.* Sample powder (0.75 g) was weighed with a precision of 0.1 mg and suspended with 8 ml of MilliQ water in polypropylene centrifugal tubes. Mixture was spiked with 200  $\mu\text{l}$  of a 5  $\mu\text{g ml}^{-1}$  [ $^{13}\text{C}_3$ ]-acrylamide methanolic solution as internal standard and later homogenized. Acrylamide extraction was performed at room temperature for 20 minutes, and 10 seconds shaking every 10 minutes. 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 (9000g; 10 min; 4°C).

*Sample clean up.* Isolute<sup>®</sup> Multimode SPE cartridges were preconditioned with 2 ml methanol, 2 ml water and 2 ml air to remove excessive water. An aliquot of the clear supernatant (1 ml) was loaded onto the cartridge at a flow rate of 2 ml  $\text{min}^{-1}$ . Then 2 ml of air were passed and finally acrylamide was eluted with 1 ml of water at the same flow rate. The solution was filtered through a 0.45  $\mu\text{m}$  filter into an amberlite LC-MS vial.

*LC-MS analysis.* Sample extracts and calibration standards were analyzed 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 (25 x 0.46 cm, 5  $\mu\text{m}$ ; Phenomenex, Torrance, CA, USA) 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  $\text{min}^{-1}$ . The injection volume was 80  $\mu\text{l}$ .

1 Electro spray ionization in the positive ionization mode was used. The MS detector  
2 operated in selected ion monitoring (SIM) mode at  $m/z$  ratios of 72.1 and 75.1 for  
3 acrylamide and [ $^{13}\text{C}_3$ ]-acrylamide respectively. Under these chromatographic  
4 conditions, acrylamide eluted at 6.8 min. A delay time of 3 min was selected to avoid  
5 the introduction of coextracted matrix components into the MS instrument prior  
6 acrylamide elution. The needle and cone voltages were set at 3.0 kV and 100 V  
7 respectively. Nitrogen was used as nebulizer gas ( $12.0 \text{ l h}^{-1}$ ) and the source temperature  
8 was set at  $300 \text{ }^\circ\text{C}$ .

9  
10 *Quantitation.* Acrylamide was quantified using a linear calibration function that was  
11 established with standard solutions of acrylamide and [ $^{13}\text{C}_3$ ]-acrylamide dissolved in  
12 Milli-Q water (25 to  $1000 \mu\text{g l}^{-1}$ ). Acrylamide contents in sample extracts were  
13 calculated from the calibration slope and intercept value, taking into account the  
14 recovery calculated by means of [ $^{13}\text{C}_3$ ]-acrylamide slope. Limit of quantification (LOQ)  
15 was  $30 \mu\text{g kg}^{-1}$  on the basis of signal-to-noise ratio of 3:1.

16  
17 *Statistical.* Statistical significance of data was tested by one-way analysis of the  
18 variance (ANOVA) (Table 5), followed by Duncan Test to compare means that showed  
19 significant variation ( $P < 0.05$ ). Analyses were performed using Statgraphics Plus,  
20 version 5.1, 2001. At least, two independent analyses were carried out per sample.

## 23 **Results and discussion**

### 24 *Sample analysis*



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1 The analysis of acrylamide was carried out by means of an analytical methodology  
2 previously established for potato chips (Rufian-Henares and Morales 2006) and  
3 subsequently adjusted for breakfast cereals (Rufian-Henares et al. 2006). The procedure  
4 was accurate, precise and had adequate sensitivity to detect the acrylamide content of  
5 the targeted food commodities

6  
7 *Bread derivatives.* A total of 45 bread types were studied: 24 crispbreads, 10 bread  
8 sticks and 11 crackers. A detailed study of acrylamide distribution was carried out, and  
9 a box-and-whisker plot was used since this graphical presentation uses a non-parametric  
10 test (Figure 1A). Acrylamide ranged from < LOQ - 151  $\mu\text{g kg}^{-1}$  for crispbread, with a  
11 mean value of 87  $\mu\text{g kg}^{-1}$  and a median of 100  $\mu\text{g kg}^{-1}$ . In the case of crackers  
12 acrylamide ranged from < LOQ - 296  $\mu\text{g kg}^{-1}$ , being the mean value 140  $\mu\text{g kg}^{-1}$  and the  
13 median 155  $\mu\text{g kg}^{-1}$ . Finally, in the case of bread sticks acrylamide ranged from <LOQ -  
14 323  $\mu\text{g kg}^{-1}$  with a mean value of 157  $\mu\text{g kg}^{-1}$  and a median of 139  $\mu\text{g kg}^{-1}$ .

15  
16 Similar values were reported by other authors (i.e. Murkovic. 2004; Senyuva and  
17 Gökmen. 2006; Yusa et al. 2006) who found acrylamide levels from 11 - 210  $\mu\text{g kg}^{-1}$  for  
18 crisp bread and, in the case of crackers values ranging from <30 - 582  $\mu\text{g kg}^{-1}$  (i.e.  
19 Riediker and Stadler, 2003; Senyuva and Gökmen. 2005). The statistical treatment  
20 showed that the acrylamide levels of crisp bread are significantly lower ( $p < 0.05$ ) than  
21 that of bread sticks and crackers. The composition of both crispbread and bread sticks is  
22 similar (mainly wheat flour, baking powder, salt and water) whereas in the case of  
23 crackers sodium hydrogencarbonate is used instead of baking powder. Taking into  
24 account these similarities, the differences in the acrylamide content could come from  
25 the different baking conditions (time and temperature) applied to the products.

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5 2 Many studies have been accomplished to find strategies to minimize the levels of  
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8 3 acrylamide. This objective can be achieved either by modifying the processing  
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10 4 parameters such as pH, temperature/time of heating or acting on precursors or key  
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12 5 intermediates. WHO and the Scientific Committee for Food of the European Union  
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15 6 called for strategies to reduce acrylamide formation to a minimum by implementing the  
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17 7 ALARA principle (as low as reasonably achievable). In this sense the German Federal  
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19 8 Office of Consumer Protection and Food Safety (BVL, 2004) stated a signal value of  
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21 9  $560 \mu\text{g kg}^{-1}$  for bread derivatives, as a concept of minimisation. Signal value is defined  
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23 10 as lowest level of the 10% containing the highest level of acrylamide. Then, if  
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25 11 acrylamide contents are above this signal value, the food producers are aware to take  
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27 12 adequate actions to lower the contents. As stated in Figure 2A, acrylamide values ranged  
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29 13 from  $< \text{LOQ} - 323 \mu\text{g kg}^{-1}$  for bread derivatives. Then, if the same concept is applied in  
30  
31 14 our survey none of the samples levels was higher than  $560 \mu\text{g kg}^{-1}$ . In this sense,  
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33 15 because of the lower values of the bread derivatives marketed in Spain, a signal value of  
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35 16  $222 \mu\text{g kg}^{-1}$  was established for the whole group. However, if the samples are divided  
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37 17 into the three original groups, the obtained signal values for crisp bread, bread sticks  
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39 18 and crackers are 129, 293 and  $232 \mu\text{g kg}^{-1}$  respectively. This is an important issue taking  
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41 19 into account that the California Environmental Protection Agency (Office of  
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43 20 Environmental Health Hazard Assessment) has proposed recently an alternative cancer  
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45 21 risk level, calculated to result in one excess case of cancer in an exposed population of  
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47 22 10 000 and assuming lifetime exposure, when consuming bread and cereals with an  
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49 23 acrylamide concentration lower than  $200 \mu\text{g kg}^{-1}$  (OEHHA. 2005).  
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1 *Biscuits*. 62 samples from 15 different producers were studied. In the case of biscuits  
2 the acrylamide content ranged from < LOQ - 2085  $\mu\text{g kg}^{-1}$ , with an average value of  
3 423  $\mu\text{g kg}^{-1}$  and a median of 268  $\mu\text{g kg}^{-1}$ . In addition, as shown in the box-and-whisker  
4 plot (Figure 1B), eight outliers were found, all of them with acrylamide contents  
5 higher than 1000  $\mu\text{g kg}^{-1}$ . Similar values have been reported by other authors (i.e.  
6 Murkovic. 2004; Senyuva and Gokmen. 2006; Yusa et al. 2006) who found acrylamide  
7 levels up to 1060  $\mu\text{g kg}^{-1}$ . When the statistical treatment was applied to the biscuits and  
8 bread derivatives groups, it was found that biscuits presents a statistically significant  
9 ( $p < 0.05$ ) higher acrylamide content. Contrary to the group of bread derivatives, the  
10 composition of biscuits is quite different and complex because different ingredients  
11 such as reducing sugars are usually added (glucose and fructose mainly added as corn  
12 syrups), fats (including  $\omega_3$ -PUFAs), technological agents (ammonium and sodium  
13 hydrogencarbonate), etc. It is well established that one of the components that promote  
14 the generation of acrylamide are reducing sugars (Friedman. 2003; Stadler et al. 2004;  
15 Zyzak et al. 2003). In addition it has been shown that the baking agent ammonium  
16 hydrogen carbonate strongly promotes the acrylamide formation (Amrein et al. 2004;  
17 Biedermann et al. 2003; Graf et al. 2006; Konings et al. 2003). Then, because of the  
18 presence of a higher content of acrylamide precursors in biscuits, their higher  
19 acrylamide content is a logical consequence.

20  
21 As described previously, biscuits comprise a heterogeneous food commodities group  
22 because of they are composed by a great variety of ingredients. Then, in order to find  
23 out the possible differences in the acrylamide content, biscuits were grouped according  
24 to the biscuit speciality, presence of ammonium hydrogen carbonate and dietary fibre  
25 content, The results of the statistical treatment are depicted in Table I. At the beginning,

1 the first analysis showed no statistical differences (data not shown) but it was found a  
2 wide distribution among groups of the outliers of Figure 2B. Then, because of their  
3 high acrylamide content the mean values of the different groups was slanted. According  
4 to the table of composition –supplied by the manufacturers- it was found that the  
5 outliers were just addressed to samples with ammonium hydrogencarbonate as the sole  
6 raising agent, in contrast with the other 54 samples where different proportions of  
7 sodium and ammonium hydrogencarbonate was used. In this sense it could be thought  
8 that a higher amount of ammonium hydrogencarbonate was used for the manufacture of  
9 the outliers and, because this raising agent enhance the acrylamide generation (Amrein  
10 et al. 2004; Biedermann et al. 2003; Graf et al. 2006; Konings et al. 2003) a larger  
11 amount of acrylamide was formed. This hypothesis explains the statistically significant  
12 differences ( $p < 0.05$ ) obtained between the group where ammonium hydrogen carbonate  
13 is the unique raising agent and those with other agents added (mean acrylamide content  
14 of 1549 and  $230.4 \mu\text{g kg}^{-1}$  respectively).

15  
16 Taking into account the explanation given above, the outliers were removed for the rest  
17 of the statistical treatment. Biscuits were classified into three groups based on the health  
18 orientation, which is closely related to their composition: those mainly used for  
19 breakfast ( $n = 21$ ), those enriched with functional ingredients ( $n = 15$ ) such as  
20 conjugated linoleic acid,  $\omega_3$ -PUFA, polyols, soy extracts, or L-carnitine, and finally  
21 those fibre enriched ( $n = 18$ ). Enriched biscuits showed significantly ( $p < 0.05$ ) lower  
22 values of acrylamide (83 vs. 247 and  $284 \mu\text{g kg}^{-1}$  for breakfast and fibre enriched  
23 biscuits respectively). It was found that most of the enriched biscuits had polyols (such  
24 as maltitol or lactitol) and potato starch replacing the addition of glucose and/or  
25 fructose. This replacement give rise to a decrease on the reducing sugar content, then

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1 not favouring the generation of acrylamide. Moreover the enriched biscuits are  
2 elaborated by adding other kind of flours such as rice or malt. Because of, in the case of  
3 breakfast cereals it has been shown that a higher potential in acrylamide formation in  
4 wheat-based cereals followed by corn, oat and rice (CIAA. 2005; Rufian-Henares et al.  
5 2006) it is plausible that the decrease on the wheat flour content reduce the acrylamide  
6 generation in biscuits.

7  
8 Biscuits usually have dietary fibre contents of about 3 – 4 %, but when enriched with  
9 wheat-bran and/or whole wheat flour, dietary fibre increases up to 10 – 30 %. As  
10 illustrated in Table 1, there were statistically significant differences in the acrylamide  
11 content between the dietary fibre added biscuits ( $283 \pm 38.1 \mu\text{g kg}^{-1}$ ;  $n = 18$ ;  
12 fibre content > 5%) and not dietary fibre-added ( $166 \pm 31.0 \mu\text{g kg}^{-1}$ ;  $n = 44$ ; fibre  
13 content < 5%), results which are in accordance to those previously reported for  
14 breakfast cereals (Rufian-Henares et al. 2006). Similar behaviour was reported by  
15 Senyuva and Gökmen (2006) who found that the acrylamide content of biscuits with  
16 dietary fibre was higher than that of the usual biscuits (486 and  $261 \mu\text{g kg}^{-1}$   
17 respectively). This could be explained by the fact that asparagine, the main precursor of  
18 acrylamide in cereal products (RHM Technologies 2005) is concentrated in the bran,  
19 particularly in wheat-bran (CIAA. 2005).

20  
21 Finally, the relationship was studied between the acrylamide content of biscuits and the  
22 signal value stated by the German Federal Office of Consumer Protection and Food  
23 Safety (BVL, 2004). This organization stated a signal value of  $575 \mu\text{g kg}^{-1}$  for adult  
24 population and  $360 \mu\text{g kg}^{-1}$  for children. As depicted in Figure 2B, when applying the  
25 same concept most of the samples (80%) are under the  $575 \mu\text{g kg}^{-1}$  level. Taking into

1 account the results in this reported survey, a signal level of  $1283 \mu\text{g kg}^{-1}$  is found  
2 although, if the eight outliers are omitted (because of the explanation given above  
3 concerning to the addition of ammonium hydrogen carbonate), a corrected level of 519  
4  $\mu\text{g kg}^{-1}$  is obtained, quite similar to that of the German Office one.

#### 6 *Acrylamide intake mediated by biscuits and bread derivatives*

7 Estimating consumer dietetic exposure to acrylamide is a high priority for governments  
8 and industry alike. Extensive research is underway to determine the extent to which  
9 acrylamide found in food is bioavailable and to identify methods to reduce levels in  
10 order to decrease consumer exposures. Consumer exposure assessments will need to be  
11 conducted in order to gauge the utility of various control options. It is particularly  
12 important to be able to assess the impact of proposed changes to the food supply  
13 including the impact of modifications in processing and cooking procedures on  
14 consumer exposures.

16 In order to estimate the dietary exposure of Spanish consumers to acrylamide from  
17 biscuits and bread derivatives, the intake of these food supplies was used (MAPA  
18 2005). The results, collected in Table II, have been expressed as  $\mu\text{g kg}^{-1} \text{ day}^{-1}$  taking  
19 into account a mean body weight of 70 kg. For total population the intake of acrylamide  
20 ranged from 0.002 - 0.058  $\mu\text{g kg}^{-1} \text{ day}^{-1}$  for crackers and biscuits respectively. In the  
21 case of biscuits, their contribution to the total daily intake of acrylamide is intermediate  
22 to that of breakfast cereals ( $0.038 \mu\text{g kg}^{-1} \text{ day}^{-1}$ ) and potato chips ( $0.093 \mu\text{g kg}^{-1} \text{ day}^{-1}$ )  
23 <sup>1)</sup> previously reported (Rufian-Henares et al. 2006; Rufian-Henares and Morales 2006).

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3 1 There are considerable differences even between many of the European countries, due  
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5 2 to the differences in food consumption patterns and cooking traditions. Irrespective of  
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7 3 the cultural differences in nutritional habits, the overall daily intake is about  $0.4 \mu\text{g kg}^{-1}$   
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9 4 day<sup>-1</sup> according to the most recent FDA estimates, basically confirming earlier  
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11 5 estimations from the US and several European authorities (Dybing et al. 2005).  
12  
13 6 Assuming a mean Spanish intake of acrylamide similar to that of other European  
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15 7 countries, a mean daily intake of acrylamide of  $0.2 \mu\text{g kg}^{-1} \text{ day}^{-1}$  can be calculated  
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17 8 assuming only the intake of the above reported foods (bread-derivatives, biscuits,  
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19 9 breakfast cereals, potato chips). Estimations of WHO of the average intake of  
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21 10 acrylamide for the general population ranged between  $0.3 - 0.8 \mu\text{g kg}^{-1} \text{ day}^{-1}$ . It was  
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23 11 supposed that children and adolescents would generally have exposures up to two to  
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25 12 three times those of adult consumers when expressed on a body weight basis (WHO,  
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27 13 2002).  
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36 15 The foods that contribute most to acrylamide exposure vary depending upon the  
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38 16 population's eating habits and the way the foods are processed and prepared. Generally,  
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40 17 the most important categories of food appear to be: fried potato products such as French  
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42 18 fries and chips, ready-to-eat breakfast cereals, baked goods such as cookies, pies and  
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44 19 cakes, brewed coffee and breads, according to the most recent results of the FDA/  
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46 20 CFSAN (Dybing et al. 2005). In this survey representative from the Spanish market  
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48 21 contribution of biscuits, crisp bread, crackers and bread sticks will be of 14.5, 4.75, 0.5  
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50 22 and 0.75% respectively of the estimated dietary intake of acrylamide. In addition the  
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52 23 contribution of potato chips and breakfast cereals will be of a 23 % and a 9.5 %  
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54 24 respectively (Rufian-Henares and Morales, 2006; Rufian-Henares et al. 2006).  
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3 1 In consumer exposure assessments it is also important to know whether there are  
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5 2 significant differences in exposures in different sub-groups of the population. In this  
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7 3 sense the exposure to acrylamide related to the those foodstuffs studied was analysed in  
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9 4 more detail taking into account socio-economic aspects (MAPA 2005). It was observed  
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11 5 that there are great differences in the intake of bread derivatives depending on the socio-  
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13 6 economic community studied, almost doubling their intake. When the number of family  
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15 7 members was analysed it could be stated that the higher intakes was in families  
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17 8 composed of 1 or 2 independent adults whereas the lower intakes where found in young  
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19 9 couples with children. In the higher cases the biscuits contribution turned from 14.5 to  
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21 10 20 % of the overall acrylamide intake. In the case of the socio-economic status the  
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23 11 exposure to acrylamide is higher in upper class due to their higher biscuit intake.  
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25 12 Contrary lower class showed higher bread derivatives intake whereas biscuits  
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27 13 consumption decreased. Finally, taking into account the population size it was found  
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29 14 that the lower acrylamide intake was recorded in cities with more than 500,000  
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31 15 inhabitants whereas the maximum intake was in localities with low-intermediate  
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33 16 number of inhabitants.  
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## 43 **Conclusions**

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45 19 This work describes the determination of acrylamide in biscuits and bread derivatives  
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47 20 cereals marketed in Spain, as a previous survey of foodstuffs had indicated these to  
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49 21 contain acrylamide. The data was used to determine the dietary intake of acrylamide for  
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51 22 the Spanish population for risk assessment purposes, it is estimated a mean daily dietary  
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53 23 intake of acrylamide of  $0.082 \mu\text{g kg}^{-1} \text{ day}^{-1}$  for which the contribution of biscuits was  
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55 24 higher than that of bread derivatives. It was also shown that in the case of biscuits,  
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57 25 higher acrylamide content was found in those manufactured with ammonium hydrogen  
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1 carbonate as the only raising agent and in those biscuits with higher proportions of  
2 dietary fibre. Conversely, lower acrylamide content was found in biscuits enriched with  
3 functional ingredients such as polyols.

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## Tables

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Table I. Statistical treatment for acrylamide levels in commercial biscuits grouped according to the biscuit speciality, presence of ammonium hydrogencarbonate as solely raising agent and dietary fibre content.

Factor	Acrylamide ( $\mu\text{g kg}^{-1}$ )	No. of samples
<i>Total</i>		
Mean	423 $\pm$ 65.6	62
Median	268	
Minimum	15	
Maximum	2085	
<i>Biscuit speciality</i>		
Breakfast	247 $\pm$ 41.8 <sup>a</sup>	21
Enriched	83 $\pm$ 37.4 <sup>b</sup>	15
Fibre added	284 $\pm$ 39.9 <sup>a</sup>	18
<i>Ammonium hydrogencarbonate</i>		
Yes	1549 $\pm$ 117 <sup>a</sup>	8
No	230 $\pm$ 36.2 <sup>b</sup>	54
<i>Dietary fibre content</i>		
< 5%	283 $\pm$ 38.1 <sup>a</sup>	18
> 5%	166 $\pm$ 31.0 <sup>b</sup>	36

<sup>a</sup> Different letters within the same factor indicate statistical differences (One-way ANOVA and Duncan test,  $p < 0.05$ )

1 Table II. Contribution of commercial bread derivatives and biscuits to the acrylamide intake<sup>a</sup> of the Spanish population.

Factor	Interval	Example	Food			
			Crisp bread	Crackers	Bread sticks	Biscuits
<i>Total population</i>			0.0190	0.0020	0.0030	0.0580
<i>Autonomic community</i>	Maximum	Extremadura for bread derivatives and Balear Islands for biscuits	0.0200	0.0030	0.0040	0.0670
	Minimum	Canary Islands for bread derivatives and Andalusia for biscuits	0.0110	0.0017	0.0020	0.0410
<i>Family components</i>	Maximum	Independent adults for biscuits and couples without children for bread	0.0230	0.0034	0.0042	0.0801
	Minimum	Young couples with children	0.0100	0.0018	0.0018	0.0418
<i>Socio-economic status</i>	Maximum	Upper class for biscuits and middle/lower class for bread derivatives	0.0235	0.0027	0.0042	0.0621
	Minimum	Upper class for bread derivatives and lower class for biscuits	0.0116	0.0016	0.0024	0.0481
<i>Population size</i>	Maximum	>2000 Inhabitants for bread derivatives and 10 000-100 000 for biscuits	0.0217	0.0027	0.0039	0.0630
	Minimum	<500 000 Inhabitants	0.0131	0.0020	0.0024	0.0480

<sup>a</sup> Results expressed as  $\mu\text{g kg}^{-1} \text{day}^{-1}$  taking into account a mean body weight of 70 kg.

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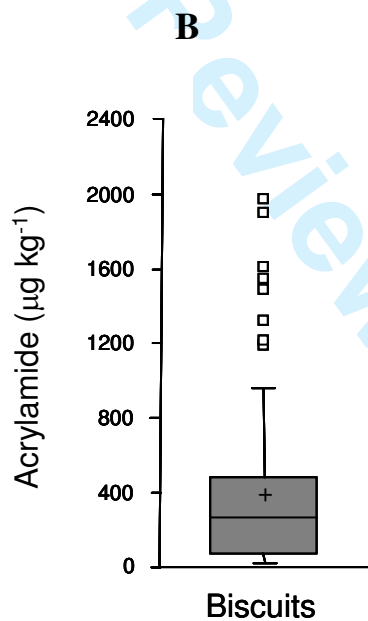
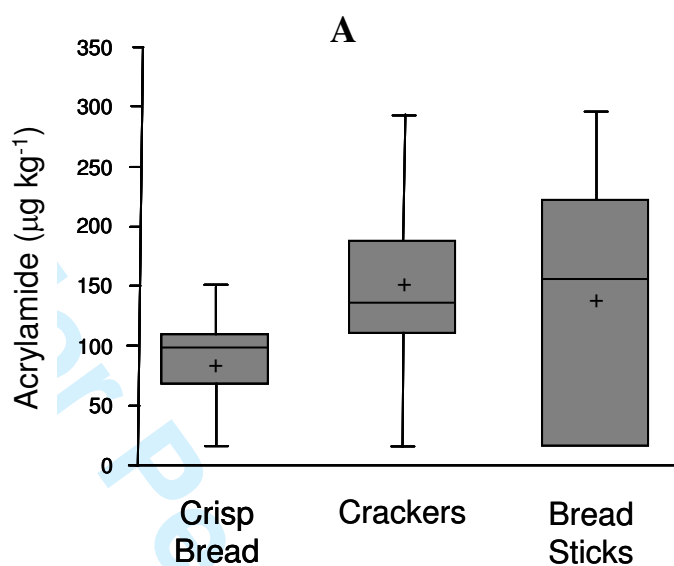
**1 Figure Captions**

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3 Figure 1. Box-and-whisker plot of acrylamide content in commercial bread derivatives  
4 (A) and biscuits (B).

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6 Figure 2. Bar graph of acrylamide content in commercial bread derivatives (A) and  
7 biscuits (B).

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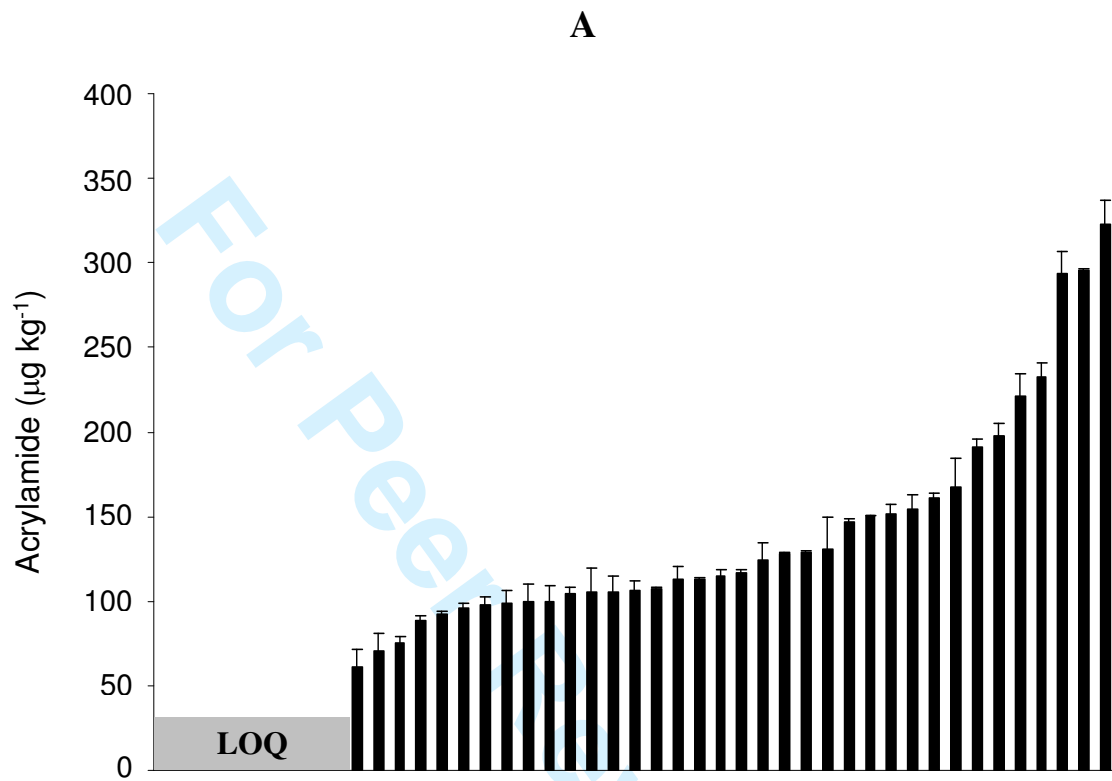
Figure 1



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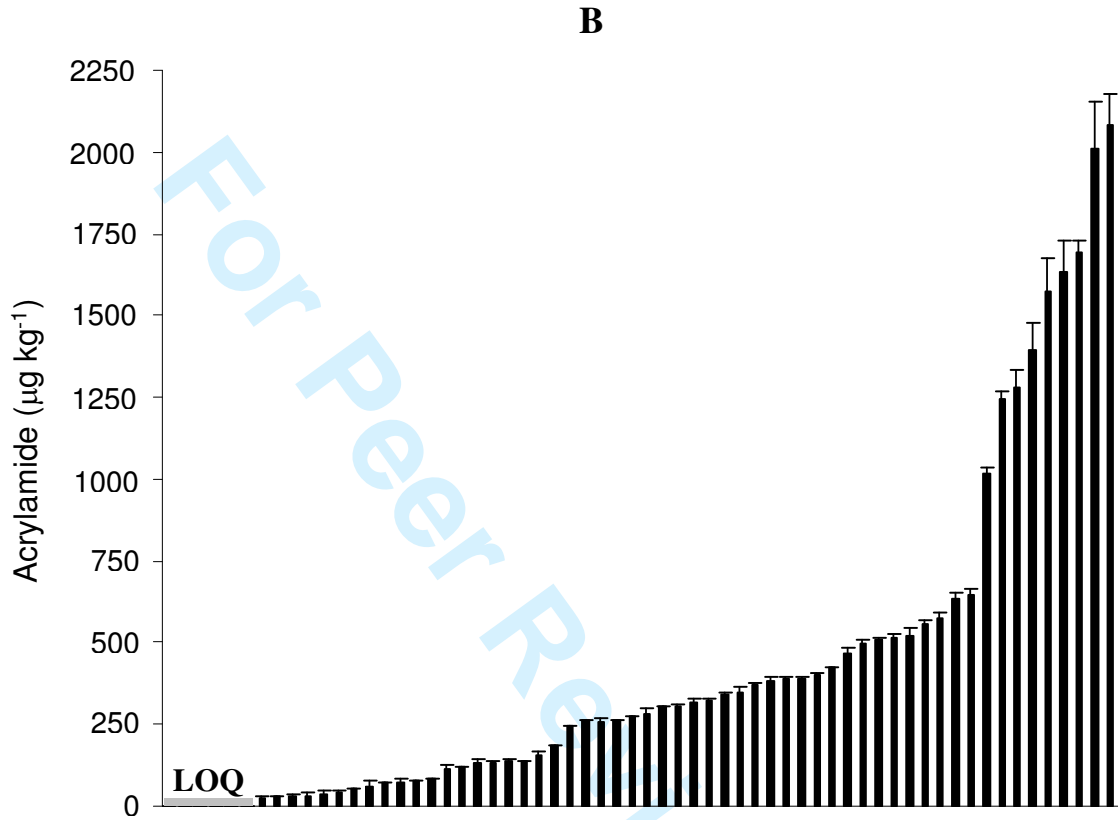
FIGURE 2A



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FIGURE 2B



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