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

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

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Abstract

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

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

Introduction

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

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

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

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

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

23
24 **Materials and methods**

25 *Samples*

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

Chemicals and materials

[¹³C₃]-acrylamide (isotopic purity 99%) was from Cambridge Isotope Labs (Andover, MA, USA). Acrylamide (99%), potassium ferrocyanide (Carrez I), 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 acetonitrile (HPLC grade) were from Scharlau (Barcelona, Spain). The solid-phase extraction (SPE) cartridges Isolute[®] Multimode (500 mg, 3 ml) were from IST (Hewgoed, Mid-Glamorgan, UK)

Acrylamide standard and reagents

Stock solutions of acrylamide (0.01 mg ml⁻¹) and [¹³C₃]-acrylamide (5 µg ml⁻¹) were prepared by dissolving the compounds in Milli-Q water and methanol respectively. These solutions were then appropriately diluted with Milli-Q water (Millipore Corp., Madrid, Spain) to prepare working standards at 1.0 µg ml⁻¹. All stock solutions and working standards were stored light-protected in a refrigerator at 4°C up to 3 months. 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.

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 μ 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 min⁻¹. The injection volume was 80 μ l.

Electrospray ionization in the positive ionization mode was used. The MS detector operated in selected ion monitoring (SIM) mode at m/z ratios of 72.1 and 75.1 for acrylamide and [$^{13}\text{C}_3$]-acrylamide respectively. Under these chromatographic conditions, acrylamide eluted at 6.8 min. A delay time of 3 min was selected to avoid the introduction of coextracted matrix components into the MS instrument prior to acrylamide elution. 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^{-1}) and the source temperature was set at 300°C .

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

Statistical. Statistical significance of data was tested by one-way analysis of the variance (ANOVA) (Table 5), 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.

Results and discussion

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 $\mu\text{g kg}^{-1}$.

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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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2 Many studies have been accomplished to find strategies to minimize the levels of

3 acrylamide. This objective can be achieved either by modifying the processing

4 parameters such as pH, temperature/time of heating or acting on precursors or key

5 intermediates. WHO and the Scientific Committee for Food of the European Union

6 called for strategies to reduce acrylamide formation to a minimum by implementing the

7 ALARA principle (as low as reasonably achievable). In this sense the German Federal

8 Office of Consumer Protection and Food Safety (BVL, 2004) stated a signal value of

9 $560 \mu\text{g kg}^{-1}$ for bread derivatives, as a concept of minimisation. Signal value is defined

10 as lowest level of the 10% containing the highest level of acrylamide. Then, if

11 acrylamide contents are above this signal value, the food producers are aware to take

12 adequate actions to lower the contents. As stated in Figure 2A, acrylamide values ranged

13 from $< \text{LOQ} - 323 \mu\text{g kg}^{-1}$ for bread derivatives. Then, if the same concept is applied in

14 our survey none of the samples levels was higher than $560 \mu\text{g kg}^{-1}$. In this sense,

15 because of the lower values of the bread derivatives marketed in Spain, a signal value of

16 $222 \mu\text{g kg}^{-1}$ was established for the whole group. However, if the samples are divided

17 into the three original groups, the obtained signal values for crisp bread, bread sticks

18 and crackers are 129, 293 and $232 \mu\text{g kg}^{-1}$ respectively. This is an important issue taking

19 into account that the California Environmental Protection Agency (Office of

20 Environmental Health Hazard Assessment) has proposed recently an alternative cancer

21 risk level, calculated to result in one excess case of cancer in an exposed population of

22 10 000 and assuming lifetime exposure, when consuming bread and cereals with an

23 acrylamide concentration lower than $200 \mu\text{g kg}^{-1}$ (OEHHA. 2005).

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Biscuits. 62 samples from 15 different producers where studied. In the case of biscuits the acrylamide content ranged from < LOQ - 2085 $\mu\text{g kg}^{-1}$, with an average value of 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 plot (Figure 1B), eight outliers where found, all of them with acrylamide contents higher than 1000 $\mu\text{g kg}^{-1}$. Similar values have been reported by other authors (i.e. Murkovic. 2004; Senyuva and Gokmen. 2006; Yusa et al. 2006) who found acrylamide levels up to 1060 $\mu\text{g kg}^{-1}$. When the statistical treatment was applied to the biscuits and bread derivatives groups, it was found that biscuits presents a statistically significant ($p<0.05$) higher acrylamide content. Contrary to the group of bread derivatives, the composition of biscuits is quite different and complex because different ingredients such as reducing sugars are usually added (glucose and fructose mainly added as corn syrups), fats (including ω_3 -PUFAs), technological agents (ammonium and sodium hydrogencarbonate), etc. It is well established that one of the components that promote the generation of acrylamide are reducing sugars (Friedman. 2003; Stadler et al. 2004; Zyzak et al. 2003). In addition it has been shown that the baking agent ammonium hydrogen carbonate strongly promotes the acrylamide formation (Amrein et al. 2004; Biedermann et al. 2003; Graf et al. 2006; Konings et al. 2003). Then, because of the presence of a higher content of acrylamide precursors in biscuits, their higher acrylamide content is a logical consequence.

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

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

Taking into account the explanation given above, the outliers were removed for the rest of the statistical treatment. Biscuits were classified into three groups based on the health orientation, which is closely related to their composition: those mainly used for breakfast ($n = 21$), those enriched with functional ingredients ($n = 15$) such as conjugated linoleic acid, ω_3 -PUFA, polyols, soy extracts, or L-carnitine, and finally those fibre enriched ($n = 18$). Enriched biscuits showed significantly ($p < 0.05$) lower values of acrylamide (83 vs. 247 and $284 \mu\text{g kg}^{-1}$ for breakfast and fibre enriched biscuits respectively). It was found that most of the enriched biscuits had polyols (such as maltitol or lactitol) and potato starch replacing the addition of glucose and/or fructose. This replacement give rise to a decrease on the reducing sugar content, then

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

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

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

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

Acrylamide intake mediated by biscuits and bread derivatives

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

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

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1 There are considerable differences even between many of the European countries, due
2 to the differences in food consumption patterns and cooking traditions. Irrespective of
3 the cultural differences in nutritional habits, the overall daily intake is about $0.4 \mu\text{g kg}^{-1}$
4 day^{-1} according to the most recent FDA estimates, basically confirming earlier
5 estimations from the US and several European authorities (Dybing et al. 2005).
6 Assuming a mean Spanish intake of acrylamide similar to that of other European
7 countries, a mean daily intake of acrylamide of $0.2 \mu\text{g kg}^{-1} \text{day}^{-1}$ can be calculated
8 assuming only the intake of the above reported foods (bread-derivatives, biscuits,
9 breakfast cereals, potato chips). Estimations of WHO of the average intake of
10 acrylamide for the general population ranged between $0.3 - 0.8 \mu\text{g kg}^{-1} \text{day}^{-1}$. It was
11 supposed that children and adolescents would generally have exposures up to two to
12 three times those of adult consumers when expressed on a body weight basis (WHO,
13 2002).
14
15 The foods that contribute most to acrylamide exposure vary depending upon the
16 population's eating habits and the way the foods are processed and prepared. Generally,
17 the most important categories of food appear to be: fried potato products such as French
18 fries and chips, ready-to-eat breakfast cereals, baked goods such as cookies, pies and
19 cakes, brewed coffee and breads, according to the most recent results of the FDA/
20 CFSAN (Dybing et al. 2005). In this survey representative from the Spanish market
21 contribution of biscuits, crisp bread, crackers and bread sticks will be of 14.5, 4.75, 0.5
22 and 0.75% respectively of the estimated dietary intake of acrylamide. In addition the
23 contribution of potato chips and breakfast cereals will be of a 23 % and a 9.5 %
24 respectively (Rufian-Henares and Morales, 2006; Rufian-Henares et al. 2006).
25

In consumer exposure assessments it is also important to know whether there are significant differences in exposures in different sub-groups of the population. In this sense the exposure to acrylamide related to the those foodstuffs studied was analysed in more detail taking into account socio-economic aspects (MAPA 2005). It was observed that there are great differences in the intake of bread derivatives depending on the socio-economic community studied, almost doubling their intake. When the number of family members was analysed it could be stated that the higher intakes was in families composed of 1 or 2 independent adults whereas the lower intakes where found in young couples with children. In the higher cases the biscuits contribution turned from 14.5 to 20 % of the overall acrylamide intake. In the case of the socio-economic status the exposure to acrylamide is higher in upper class due to their higher biscuit intake. Contrary lower class showed higher bread derivatives intake whereas biscuits consumption decreased. Finally, taking into account the population size it was found that the lower acrylamide intake was recorded in cities with more than 500,000 inhabitants whereas the maximum intake was in localities with low-intermediate number of inhabitants.

Conclusions

This work describes the determination of acrylamide in biscuits and bread derivatives cereals marketed in Spain, as a previous survey of foodstuffs had indicated these to contain acrylamide. The data was used to determine the dietary intake of acrylamide for the Spanish population for risk assessment purposes, it is estimated a mean daily dietary intake of acrylamide of $0.082 \mu\text{g kg}^{-1} \text{ day}^{-1}$ for which the contribution of biscuits was higher than that of bread derivatives. It was also shown that in the case of biscuits, higher acrylamide content was found in those manufactured with ammonium hydrogen

carbonate as the only raising agent and in those biscuits with higher proportions of dietary fibre. Conversely, lower acrylamide content was found in biscuits enriched with functional ingredients such as polyols.

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Tables

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 ± 65.6	62
Median	268	
Minimum	15	
Maximum	2085	
<i>Biscuit speciality</i>		
Breakfast	247 ± 41.8^a	21
Enriched	83 ± 37.4^b	15
Fibre added	284 ± 39.9^a	18
<i>Ammonium hydrogencarbonate</i>		
Yes	1549 ± 117^a	8
No	230 ± 36.2^b	54
<i>Dietary fibre content</i>		
< 5%	283 ± 38.1^a	18
> 5%	166 ± 31.0^b	36

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

Table II. Contribution of commercial bread derivatives and biscuits to the acrylamide intake^a 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

^a 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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4 1 **Figure Captions**

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8 3 Figure 1. Box-and-whisker plot of acrylamide content in commercial bread derivatives
9 4 (A) and biscuits (B).

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11 5

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13 6 Figure 2. Bar graph of acrylamide content in commercial bread derivatives (A) and
14 7 biscuits (B).

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Figure 1

FIGURE 2A

FIGURE 2B