LWT - Food Science and Technology 46 (2012) 16–22

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Assessment of acrylamide intake of Spanish boys aged 11–14 years consuming a traditional and balanced diet

Cristina Delgado-Andrade [a,*], Marta Mesías [b], Francisco J. Morales [c], Isabel Seiquer [a], M. Pilar Navarro [a]

[a] Unidad de Nutrición (IFNA), EEZ-CSIC, Camino del Jueves, 18100 Armilla, Granada, Spain
[b] Departamento de Nutrición y Bromatología, Facultad de Farmacia, University of Granada, Granada, Spain
[c] Instituto de Ciencia y Tecnología de los Alimentos y Nutrición, ICTAN- CSIC, Madrid 28040, Spain

ARTICLE INFO

Article history:
Received 23 November 2010
Received in revised form
4 November 2011
Accepted 10 November 2011

Keywords:
Acrylamide
Intake
Traditional diet
Adolescents

ABSTRACT

Acrylamide is a toxic and potentially cancer causing chemical formed in thermally treated foods, especially in carbohydrate rich. New dietary habits acquired by adolescents, increasing snacking and fast food consumption, lead to a high acrylamide intake, since the composition of these meals and their processing promote its appearance. This study investigated acrylamide daily exposure in a group of Spanish male adolescents (11–14 years) consuming a balanced diet based on their food preferences but reducing snacking and fast food, aimed to determine whether acrylamide exposure was reduced by the consumption of a traditional diet. Acrylamide content of main dishes was analyzed and input per serving calculated. The Spanish potato omelette and the grilled loin of pork with fried potatoes presented the highest levels of acrylamide (128 and 111 μg/kg respectively), followed by different meals also containing potatoes or cereal derived products. The acrylamide total intake was estimated at 0.534 μg/kg body weight/day, where the highest percentage was provided by the breakfast (31.66%), the afternoon snack being the lowest contributor (16.19%). These results indicate consumption of a balanced and traditional diet, besides the well known beneficial effects on health, can also moderate acrylamide exposure and thus its possible long-term toxicological effect.

1. Introduction

Since acrylamide was identified in April 2002 as a heat-induced process contaminant in fried, baked, grilled or toasted carbohydrate-rich foods (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002) with demonstrated genotoxic and carcinogenic effects in animal experiments (European Commission, 2000; IARC, 1994), the estimation and prevention of consumer exposure to this compound has been one of the highest priorities for governments and industry. The Maillard reaction of asparagine and reducing sugars is considered its most probable mechanism of formation (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002), although contribution of lipid oxidation products in fatty rich foods is relevant (Zamora & Hidalgo, 2008). Moreover, it is well-established that processing conditions, such as time, temperature and food matrix, dramatically influence acrylamide formation (Friedman, 2003).

In order to set the potential risk to population, acrylamide dietary exposure has been evaluated in different countries, populations and dietary habits (Alexander, 2006). Monitoring studies carried out by European Commission from 2007 show that acrylamide content in food ranges from less than 30 to 4700 μg/kg depending on product type (EFSA, 2010). Worldwide acrylamide intakes ranged from 0.3 to 2.0 μg/kg body weight (bw)/day for average consumers and from 0.6 to 5.1 μg/kg bw/day for high consumers (90th to 99th percentiles) (FAO/WHO, 2005). In addition, deterministic- and probabilistic approaches have been carried out for exposure assessment. However most evaluations have focussed in foods considered the most significant contributors, such as processed cereals and potatoes, coffee, snacks, etc., and by then applying the food consumption data supplied by official institutions. But food consumption is grouped by food categories, usually as raw and sometimes as industrially processed foods. The problem is that raw foods are often submitted to culinary processes before consumption, during which acrylamide formation, or even destruction, may take place. This second source (traditional cooking) is often omitted from probabilistic studies; nevertheless, for a reliable calculation, both sources of acrylamide inputs to the diet must be considered.

In the population as a whole, acrylamide intake is strongly affected by age (Konings et al., 2003; Saleh & El-Okazy, 2007). The older population tends to consume less acrylamide, as their

* Corresponding author. Tel.: +34 958 572757; fax: +34 958 572753.
E-mail address: cdelgado@eez.csic.es (C. Delgado-Andrade).

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doi:10.1016/j.lwt.2011.11.006

consumption of high-temperature processed food is lower. On the contrary, the adolescent population is the group most likely to have a high acrylamide intake, due to their dietary habits, such as high levels of snacking (essentially cereal and potato derived products) and fast food consumption, in which frying, roasting, grilling, baking and even reheating before consumption are commonly applied. These conditions, joined to the particular composition of raw materials, tend to maximize acrylamide formation and thus its consumption. In addition, children and teens have lower average body weight than adult and, consequently, a higher average food intake per kilogram of bw.

In today's society of developed countries, government agencies seek to raise awareness of the importance of reducing snacking and maintaining the traditional diet, for a healthy life, especially in the young people, among whom the indices of obesity and cardiovascular disease are becoming alarming.

In this context, the goal of this study was to assess the acrylamide daily exposure in a group of Spanish male adolescents aged 11–14 years consuming a balanced diet based on their food preferences but reducing snacking and fast food, in order to determine whether maintaining a traditional diet moderates acrylamide exposure in this population, besides providing the known beneficial effects on health. The study was focused on this population due its dietary habits and to the fact that this is the time when alimentary behaviours that will keep the rest of life are becoming. In parallel, the acrylamide content of the main dishes in the diet was analyzed, and the contribution of each meal (breakfast, lunch, afternoon snack and dinner) to the daily acrylamide intake was established.

2. Materials and methods

2.1. Chemicals

Potassium ferrocyanide, zinc acetate and formic acid were purchased from Panreac (Barcelona, Spain). $^{13}C_3$-Acrylamide (isotopic purity 99%) was obtained from Cambridge Isotope Labs (Andover, MA, USA). Ultra pure water was used throughout the experiments (Milli-Q system, Millipore, Bedford, MA, USA).

2.2. Subjects, diets and study design

Twenty male adolescents aged 11–14 years (12.4 $\pm$ 0.34 years, mean $\pm$ SE), weighing 55.9 $\pm$ 2.9 kg, participated in a 2-week trial, in which they consumed a designed diet based on their eating patterns, previously evaluated by nutritional survey, and following the Recommended Intakes for the Spanish Population (Moreiras, Carbajal, Cabrera, & Cuadrado, 2004), aimed to compose a balanced diet. A 7-day lunch and dinner menu was created. Both meals were prepared daily by a local catering firm, always based on the traditional recipes of the domestic cooking (Table 1) and were distributed to the homes of the participants. The 7-day menu was repeated twice during the 14-day experimental period. Breakfast and the afternoon snack were consumed at home for all the participants, choosing from a wide range of allowed foods. The foods included in breakfast were: milk, milk shakes, fruit juices, sandwich bread, margarine, marmalades, cocoa powder, chocolate cream, cake, breakfast cereals, doughnuts and chocolate biscuits. The afternoon snack was composed of milk, milk shakes, fruit juices, sandwich bread, mortadella, pâté, biscuits, cocoa powder, doughnuts, chocolate doughnuts, popcorn, cake, milk or dark chocolate, chocolate turrón (a Spanish product typically consumed at Christmas), chocolate biscuits and different salty snacks; these are described in Table 2. The food composition of both meals was recorded daily by questionnaire. It must be noted that some of the participants also made a midmorning snack, compounded by the same foods described as breakfast and afternoon snack. In those cases, the midmorning snack was considered within the breakfast to perform the calculations of dietary acrylamide exposure.

The food composition of the diet was transformed into energy and nutrient values using the Spanish Food Composition Tables (Mesías, Seiquer, Delgado-Andrade, Galdó, & Navarro, 2009), under AYS44 Diet Analysis software supplied by ASDE, SA (Valencia, Spain). Compliance with dietary treatments was assessed by daily record sheets, in which the participants noted the details of their food consumption and recorded all the food that was left to be taken into account. The above-mentioned software was also used to obtain energy and nutrient intake from the food consumption data. The overall daily intake was as follows: energy 2324 $\pm$ 45 kcal, fat 94.2 $\pm$ 1.9 g, carbohydrate 279.0 $\pm$ 5.8 g, protein 82.4 $\pm$ 1.9 g, fibre 21.2 $\pm$ 0.6 g, cholesterol 239.0 $\pm$ 8.7 mg, calcium 929 $\pm$ 19 mg, phosphorus 1320 $\pm$ 26 mg, magnesium 291 $\pm$ 9 mg, iron 14.6 $\pm$ 0.4 mg, zinc 7.7 $\pm$ 0.2 mg, retinol 1.00 $\pm$ 0.03 mg, ascorbic acid 97 $\pm$ 5 mg, α-tocopherol 9.7 $\pm$ 0.4 mg.

To enable analysis of the acrylamide content in the experimental diet, the catering firm also provided the lunch and dinner meals to the researchers in order to analyze the acrylamide content in the edible portion. The acrylamide contained in the food of the breakfast and afternoon snack were obtained from bibliographic

Table 1
Lunch and dinner 7-d menus for the dietary treatment.

MEAL	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Lunch	Lentil stew	Sauté vegetables	Spanish omelette with ham	Legume (beans) and rice stew	Paella	Fried chicken and fried potatoes	Tropical salad
	Empanadillas[a] with salad (lettuce, tomato, etc)	Griddle loin of pork and fried potatoes	Meatballs (veal) with vegetables	Salad (lettuce, tomato, tuna, etc)	Salad (lettuce, tomato, tuna, etc)	Salad with tuna (lettuce, tomato, etc)	Spanish potato omelette
	Chocolate yoghurt	Torrija[b]	Chocolate custard	Chocolate yoghurt	Apple	Pear	Rice with milk and cinnamon
	Bread	Bread	Bread	Bread	Bread	Bread	Bread
Dinner	Consommé with noodles	Gratin macaroni with béchamel sauce	Soup of vegetables	Soup of pasta and chicken	Vegetables cream with croutons	Purée of prawns	Breaded fish and boiled rice
	Breaded fish with fried potatoes	Banana	Pizza	Hamburger with fried potatoes	Breaded hake fish-fingers	Fish croquettes and fried potatoes	Mandarins
	Pears	Bread	Banana	Banana	Chocolate custard	Caramel custard	Bread
	Bread		Bread	Bread	Bread	Bread	

[a] Small tuna-filled breaded pasties.
[b] Fried bread with milk, sugar and cinnamon.

Table 2
Acrylamide content in dishes constituting the lunch and dinner 7-d menus and in the foods consumed for breakfast and the afternoon snack.

	Acrylamide (μg/kg dry matter)[a]	Mean serving size (g)	Acrylamide (μg/serving)	Source
Dishes for lunch and dinner				
Lentil stew	nd	457	–	Analyzed
Empanadillas[b] with salad	nd	241	–	Analyzed
Chocolate yoghurt	nd	98	–	Analyzed
Bread	130	40	5.2	FDA, 2006
Consommé with noodles	5	443	0.2	Analyzed
Breaded fish with fried potatoes	28	208	2.5	Analyzed
Pears	nd	139	–	Analyzed
Sauté vegetables	nd	367	–	Analyzed
Griddle loin of pork and fried potatoes	111	230	10.3	Analyzed
Torrija[c]	16	154	1.5	Analyzed
Gratin macaroni with béchamel	nd	338	–	Analyzed
Banana	nd	85	–	Analyzed
Spanish omelette with ham	36	219	2.4	Analyzed
Meatballs (veal) with vegetables	14	236	1.0	Analyzed
Chocolate custard	nd	137	–	Analyzed
Vegetable soup	5	400	0.20	Analyzed
Pizza	20	101	2.0	OEHHA, 2005
Legume (beans) and rice stew	22	488	2.4	Analyzed
Salad	nd	201	–	Analyzed
Pasta and chicken soup	nd	443	–	Analyzed
Hamburger with fried potatoes	14	197	1.1	Analyzed
Paella	17	322	2.2	Analyzed
Apple	nd	125	–	Analyzed
Vegetable cream with croutons	20	421	0.8	Analyzed
Breaded hake fish-fingers	16	202	1.6	Analyzed
Fried chicken and fried potatoes	11	207	1.2	Analyzed
Fish croquettes and fried potatoes	59	361	8.1	Analyzed
Caramel custard	nd	147	–	Analyzed
Tropical salad	nd	191	–	Analyzed
Spanish potato omelette	128	251	6.4	Analyzed
Rice with milk and cinnamon	nd	163	–	Analyzed
Breaded fish and rice	22	224	2.1	Analyzed
Mandarins	nd	123	–	Analyzed
Foods included in breakfasts and afternoon snacks				
Milk	nd	200	–	OEHAA, 2005
Milk shake	nd	200	–	OEHAA, 2005
Fruit juice	nd	200	–	OEHAA, 2005
Mortadella	nd	50	–	OEHAA, 2005
Margarine	nd	15	–	OEHAA, 2005
Marmalade	nd	15	–	OEHAA, 2005
Pâté	nd	15	–	OEHAA, 2005
Sandwich bread	11	20	0.2	OEHAA, 2005
Biscuits	426	45	21.3	Rufián-Henares, Arribas-Lorenzo, & Morales, 2007
Cocoa powder	50	10	1.0	FDA, 2006
Chocolate doughnut	23	55	2.3	Sadd & Hamlet, 2005
Chocolate cream	25	15	0.2	OEHAA, 2005
Popcorn	290	100	8.7	Eerola, Hollebekkers, Hallikainen, & Peltonen, 2007
Cake	20	80	1.2	OEHAA, 2005
Breakfast cereals	292	30	14.6	Rufián-Henares, Delgado-Andrade, & Morales, 2006
Milk chocolate	125	200	3.8	Ren, Zhang, Jiao, Cai, & Zhang, 2006
Chocolate turrón[d]	133	35	2.0	Bermudo et al., 2008
Tortilla chips	486	30	7.3	Zubeldia & Gomar, 2007
Potato crisps	500	50	15.0	Zubeldia & Gomar, 2007
Crisp corn snack	194	30	3.9	Zubeldia & Gomar, 2007
Flaked rice snack	56	30	2.8	OEHAA, 2005
Doughnut	18	45	0.8	OEHAA, 2005
Sunflower seeds	40	15	0.8	OEHAA, 2005
Peanuts	27	20	0.5	OEHAA, 2005
Dark chocolate	190	20	4.8	Mestdagh et al., 2007
Chocolate biscuits	130	45	6.5	OEHAA, 2005

Nd: not detectable; value below the limit of detection (<3 μg/kg). Hyphen indicates inappreciable amount of the compound in a normal serving.
[a] $N = 2$ for samples analyzed in our facilities.
[b] Small tuna-filled breaded pasties.
[c] Fried bread with milk, sugar and cinnamon.
[d] A typical Spanish Christmas product based on chocolate, flaked rice, honey, sugar and vegetable fats.

sources. Since this was a Spanish study, bibliographic data coming from Spanish food were used as far as possible. When the acrylamide level was not documented in one of our particular foods, the data applied was that we considered that could come closer to the Spanish situation.

This study was approved by the Ethics Committee of the San Cecilio University Hospital of Granada and was performed in accordance with the Helsinki Declaration of 2002, as revised in 2004. The informed consent was obtained from the parents of all the children participating in the study.

2.3. Liquid chromatography-electrospray ionization single quadrupole mass spectrometry (LC–ESI-MS) determination of acrylamide

Sample preparation was as described by Arribas-Lorenzo and Morales (2009). A finely ground sample (0.450 g) was then weighed into a 10 ml centrifuge tube. The sample was spiked with 100 μl $^{13}C_3$-labelled acrylamide (10 μg/ml) for recovery, and 5 ml of Milli-Q water were added. The mixture was then vortexed, kept for 5 min at room temperature and subsequently homogenized for 1 min (Ultraturrax). Then, 750 μl Carrez I (potassium ferrocyanide, 15 g/100 ml) and 750 μl Carrez II (zinc acetate, 30 g/100 ml) solution were added, vortexed and left to stand for 10 min. The tubes were then centrifuged at 9000 g for 15 min at 4 °C. The clear supernatant (1 ml) was clarified onto a pre-conditioned Oasis-HLB cartridge (30 mg, 1 ml, Waters, Milford, MA, USA). The first drops were discharged and the rest of the eluate was collected in amberlite vials for analysis. LC–ESI-MS analyses were performed using an Agilent 1100 high-performance liquid chromatography (HPLC) system (Waldbronn, Germany) consisting of a quaternary pump, an autosampler and a temperature-controlled column oven, coupled to an Agilent 1100 MS detector equipped with an electrospray ionization interface. The analytical separation was performed on an Inertsil ODS-3 column (250 × 4.6 mm, 5 μm; GL Sciences Inc, Tokyo, Japan) using an isocratic mixture of 0.2 ml/100 ml aqueous solution of formic acid at a flow rate of 0.6 ml/min at 25 °C. Data acquisition was performed, with a delay time of 8 min, in a selected ion-monitoring (SIM) mode using the following interface parameters: a drying gas (N_2, 665 Pa) flow of 12 l/min, nebulizer pressure of 300 Pa, drying gas temperatures of 350 °C, a capillary voltage of 3 kV and a fragmenter voltage of 70 eV. Monitored ions were 72.1 m/z for acrylamide and 75.1 m/z for $^{13}C_3$-labelled acrylamide. Standard solutions of acrylamide and [$^{13}C_3$]-acrylamide dissolved in Milli-Q water were used to build a linear calibration curve in the range of 1–100 μg/l. The limit of detection was 3 μg/kg and the limit of quantification was 10 μg/kg. The method was in-house validated for linearity, precision and recovery. Furthermore, the accuracy was demonstrated for potato crisps, crispsbread, and biscuit in proficiency tests carried out by Institute for Reference Materials and Measurements (IRMM) and FAPAS, yielding satisfactory z-scores between 0.5 and 1.0. Precision (reproducibility) was lower than 12% and recovery was between 84 and 109%. The analyses are integrated within the scope of a certified laboratory monitored by AENOR (the Spanish Association for Standardization and Certification).

2.4. Exposure assessment

In order to calculate the dietary intake of acrylamide in the twenty male adolescents a deterministic approach was developed. Acrylamide level in dishes compounding lunch and dinner were analyzed as described previously. For each dish, the contribution to dietary acrylamide intake was estimated from the mean acrylamide level in the dish and the amount consumed by each participant (estimated by daily record sheets). When not detectable level of acrylamide was detected in a dish (<3 μg/kg), it was considered that its contribution to the acrylamide intake was null.

Since breakfast and afternoon snack were free, choosing from a wide range of allowed foods, the items consumed and the amounts were recorded daily by questionnaire. To calculate the dietary acrylamide intake from those bibliographic sources were applied. The use of bibliographic data coming from Spanish food was prioritary, but if the acrylamide level was not documented in one of our particular foods, the data applied was that we considered that could come closer to the Spanish situation.

3. Results and discussion

3.1. Acrylamide content in dishes constituting the lunch and dinner 7-d menus

Table 2 presents the data for acrylamide content in the dishes of the designed diet. The highest acrylamide concentrations were found in the Spanish potato omelette (128 μg/kg dry matter) followed by the grilled loin pork and fried potatoes (111 μg/kg dry matter). However, this order is inverted when the intake of acrylamide per serving is considered (6.4 and 10.3 μg/serving for the omelette and loin, respectively), due to the greater moisture content in the potato omelette. The presence of fried potatoes in both dishes could be the main factor responsible for the high levels detected, since this food has been described as one of the largest contributors to acrylamide intake (Arribas-Lorenzo & Morales, 2009). The high acrylamide level in the Spanish potato omelette was reported by our research group in a previous study of different Spanish dishes (Delgado-Andrade, Morales, Seiquer, & Navarro, 2010). However, it must be noted that other dishes containing fried potato like breaded fish, hamburger or fried chicken did not reach such values of acrylamide. A possible explanation for this result could be the use of different potato varieties. Amrein et al. (2003) concluded that acrylamide contents in potato products can be substantially reduced primarily by selecting cultivars with low concentrations of reducing sugars.

Dishes containing rice submitted to culinary processes involving a higher heat charge, such as stir frying, also showed a significant acrylamide content, and thus also of intake per serving. These were the cases of paella or legume (beans) and rice stew. However, other legume stews without rice, such as lentils, did not present detectable amounts of the compound. When the rice was submitted to boiling, such as rice with milk and cinnamon, acrylamide formation was not induced, probably due to the high moisture content of the preparation given by the milk. In this sense, apart from reaction time and temperature, it has been reported that the major difference between thermal procedures leading to high or low amounts of acrylamide from asparagine is the water content of the reaction system, since the water activity has been shown to be a key factor to consider in the Maillard reaction (Ciesarová, Kiss, & Kolek, 2006; Robert et al., 2005).

In fried and breaded foods, acrylamide was present, since wheat is also an adequate substrate of formation, as shown in the bread in the diet (130 μg/kg dry matter, based on FDA determinations, 2006). Thus, dishes where a low content of acrylamide was expected, due to the major presence of vegetables, depicted noteworthy levels when bread-derivates were added, as in the case of the vegetable cream with croutons (20 μg/kg dry matter), although this dish was not a major contributor to the intake of the compound (0.8 μg) due to the high water content of the serving. For this same reason, the soups included in the diet did not contribute significantly to the acrylamide intake (0.2 μg/serving for both the consommé with noodles and the vegetable soup, while the intake of the compound was inappreciable for the pasta and chicken soup).

As is logical since no thermal treatment was applied, the acrylamide content in fresh fruits was not detectable and the latter did not contribute to acrylamide input to the diet.

Generally, among the dishes in the lunch and dinner 7-day menus, if proper reactants were present, frying was the culinary process that presented the highest rate of formation of acrylamide, as reported in a previous study of dishes in the Spanish diet (Delgado-Andrade et al., 2010). Other authors have also demonstrated that frying was the most promoting process for acrylamide formation (Carrieri, Anese, Quarta & De Bonis, Ruocco, 2010). The values reported in the present study were lower than those found by Bermudo, Moyano, Puignou, and Galceran (2008) in different

Spanish food products, probably because the composition of the foods selected by the latter authors was particularly liable to acrylamide formation, while in our case a complete, balanced diet was considered.

Among the foods included by the participants in the breakfast and afternoon snack, the industrially processed ones based on potato or cereals were the largest contributors to the daily acrylamide intake, such as biscuits (with or without chocolate), popcorn, breakfast cereals, tortilla chips or potato crisps. In the same line, the study by Mestdagh et al. (2007) also established that biscuits, canteen French fries, bread and chocolate were the major contributors to daily acrylamide intake.

3.2. Acrylamide exposure

The participants' acceptance of the diet was satisfactory and the differences between the lunch and dinner acrylamide intakes were due to the fact that, in some cases, not all of the serving was consumed; in this case, the values for the leftovers were subtracted (Table 3). The main meals of the adolescents' diet provided a mean acrylamide intake of 29.83 μg/day, ranging from 15.39 to 50.17 μg/day with a median value of 27.67 μg/day. 50% of the subjects presented an acrylamide intake between 22.92 and 36.43 μg/day. Breakfast, lunch and dinner were the meals providing the largest contribution to the daily acrylamide input. In the case of lunch and dinner, the values varied little among subjects, since these meals were supplied by the local catering firm as designed by the researchers. Thus, with respect to lunch, a median value of 8.57 μg/day of acrylamide was consumed, with half of the subjects receiving between 8.11 and 8.86 μg/day during the experimental period. In this meal, only five boys consumed acrylamide levels above the median value. Similar results were obtained for the dinner, in which the median acrylamide intake was 7.53 μg/day and 50% of the subjects consumed between 7.25 and 7.80 μg/day.

However, the acrylamide input from breakfast and the afternoon snack was more irregular because of the wide variety of foods chosen by the participants, according to their preferences, although snacking was reduced (only six boys in this study declared the additional consumption of salty snacks). For this reason, acrylamide values ranged from 0.35 to 26.86 μg/day for the breakfast and from 0 to 17.60 μg/day for the afternoon snack.

Table 3
Daily acrylamide intake during the 14-d experimental period.

Subject	Global (μg/day)	Breakfast (μg)	Lunch (μg)	Afternoon snacks (μg)	Dinner (μg)
1	39.34	20.65	8.99	1.86	7.84
2	22.26	1.00	8.82	4.60	7.84
3	47.75	26.86	8.64	3.55	8.71
4	15.39	0.35	7.55	0.00	7.49
5	18.48	0.79	8.78	1.39	7.53
6	28.44	10.91	7.78	2.55	7.23
7	36.61	2.62	7.75	17.60	7.81
8	22.44	6.53	8.57	8.23	3.85
9	31.44	12.99	3.83	1.74	7.82
10	25.46	4.48	8.90	4.71	7.53
11	27.38	9.35	8.74	1.23	7.89
12	50.17	20.24	8.91	13.50	7.35
13	36.82	11.52	9.08	9.40	7.34
14	27.04	4.71	8.56	7.48	6.41
15	27.95	15.70	8.44	0.00	4.03
16	24.36	6.07	8.22	1.43	8.66
17	36.25	18.82	8.20	1.62	7.33
18	33.51	6.57	8.47	10.20	7.83
19	23.39	4.00	8.91	2.83	8.54
20	22.13	4.73	8.02	2.66	6.96
Mean	29.83	9.44	8.26	4.83	7.30
SE	2.05	1.70	0.25	1.07	1.27
Median	27.67	6.55	8.57	2.72	7.53
Maximum	50.17	26.86	9.08	17.60	8.71
Minimum	15.39	0.35	3.83	0.00	3.85
Lower quartile	22.92	4.24	8.11	1.53	7.28
Upper quartile	36.43	14.35	8.86	7.86	7.84

As proof that snacking was reduced during the experimental period, it was observed that in the afternoon snack, in which the highest amount of snacking tends to occur, the percentage of acrylamide input was lower than for the breakfast (16.19% *vs.* 31.66%, respectively) (Fig. 1). In the study by Mestdagh et al. (2007), the afternoon snack was not considered, but participants consumed a midmorning snack which constituted 42.2% of the daily acrylamide input, mainly contributed by biscuits. This value is much higher than the percentage of acrylamide contributed by the afternoon snack, and even by breakfast, calculated in the present study.

Lunch and dinner represented similar percentages of acrylamide intake (27.68% and 24.47%, respectively). The contribution reported by Mestdagh et al. (2007) for both meals was lower (15.5 and 19.1%, respectively), which thus highlights the major importance of these meals among the Spanish population.

The mean daily acrylamide consumption found in the present assay was 0.534 μg/kg bw/day, which is in the same order of magnitude as has been found for other European countries such as Germany, Norway, ~~France~~, Sweden and Belgium, in studies carried out in different population segments, and well below the values measured in Alexandria (Egypt), Poland and some of the studies in the Netherlands (Fig. 2). In addition, our findings are in agreement with the long-term dietary acrylamide exposure in developed countries, as calculated by the FAO/WHO (2002), of between 0.3 and 0.8 μg/kg bw/day. The value estimated is also lower than the level of 40 μg/kg bw/day, considered to be the tolerable daily intake (TDI) for neurotoxicity of acrylamide, and lower than the range of 2.6–16 μg/kg bw/day proposed for the development of some types of cancer (Tardiff, Gargas, Kirman, Carson, & Sweeney, 2010). However, it should be noted that in the last evaluation carried out by JECFA (Joint FAO/WHO Expert Committee on Food Additives) in 2010, acrylamide was still considered a genotoxic carcinogen for which no safe levels could be established.

The daily acrylamide exposure established in the present study exceeds that indicated by Morales (unpublished, 2004) for the whole population, reported in Arribas-Lorenzo and Morales (2009). The biggest difference between the two estimations is the fact that Morales' values were based on the determination of acrylamide only in foods regarded as major contributors, mainly resulting from industrial processing (coffee, chips, and biscuits, among others). After the analysis, consumption data for the Spanish population, as determined by the Ministry of Agriculture, Fisheries and Food, was

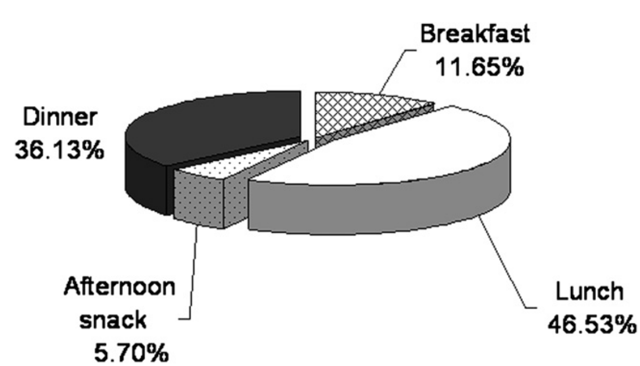

Fig. 1. Mean distribution of acrylamide intake in the four daily meals considered, expressed as percentage of the daily acrylamide exposure.

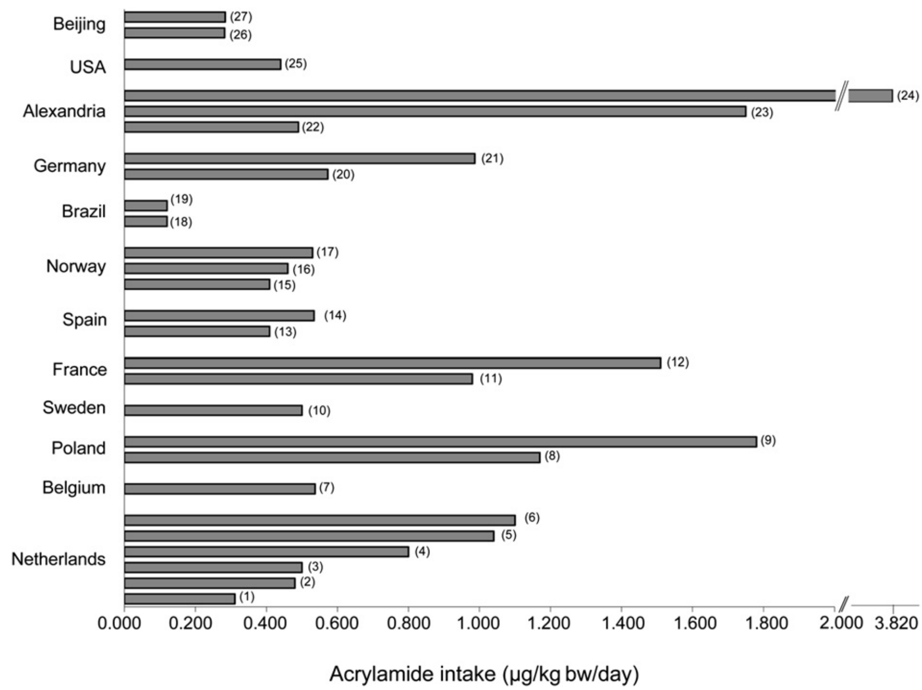

Fig. 2. Dietary acrylamide exposure in different studies and countries. (1) Schouten, Hogervost, Konings, Goldbohm, and Van den Brandt (2009), whole population aged 55–69 years. (2) Konings et al. (2003), whole population aged 1–97 years. (3) Boon et al. (2005), whole population aged 1–97 years. (4) Konings et al. (2010), whole population aged 18–74 years. (5) Konings et al. (2003), young population aged 7–18 years. (6) Boon et al. (2005), children aged 1–6 years. (7) Mestdagh et al. (2007), young adults aged 18–35 years. (8) Gielecinska et al. (2007), adolescents aged 14–18 years. (9) Gielecinska et al. (2007), children aged 7–13 years. (10) Svensson et al. (2003), whole population aged 18–74 years. (11) Agence Française de Sécurité Sanitaire des Aliments (AFSSA) (2005), children aged 9–14 years. (12) AFSSA (2005), children aged 3–8 years. (13) Morales (unpublished, 2004) whole population, in Arribas-Lorenzo and Morales (2009). (14) Present study (2010), male adolescents aged 11–14 years. (15) Dybing and Sanner (2003), females aged 6–79 years. (16) Dybing and Sanner (2003), males aged 16–79 years. (17) Dybing and Sanner (2003), males aged 10–30 years. (18) Arisseto et al. (2009), adolescents aged 11–14 years. (19) Arisseto et al. (2007), adolescents aged 11–17 years. (20) Mosbach-Schulz et al. (2003), whole population aged 19–64. (21) Mosbach-Schulz et al. (2003), children aged 7–14. (22) Saleh and El-Okazy (2007), whole population aged >50 years. (23) Saleh and El-Okazy (2007), whole population. (24) Saleh and El-Okazy (2007), children aged 3–6 years. (25) Food and Drug Administration (FDA) (2006), whole population aged >2 years. (26) Qing-Zhong, Li-Wen, Jun, Wei, and Ren-Cai (2006), males all ages. (27) Qing-Zhong et al. (2006), females all ages.

applied to calculate the acrylamide intake. On the contrary, the present test involves examination of the contribution made by the main by high, since the diet provided is balanced and varied, with a significant content of fresh fruits and vegetables, which logically helps to reduce the acrylamide intake, as reported by Mestdagh et al. (2007).

However, we recognize that our designed diet differs at some aspects from that normally consumed by Spanish adolescents, in the sense that young people in Spain have a higher snack and fast food consumption than was the case of the study subjects. This fact is not unique to Spain, but is common in many developed countries. As a consequence, the daily acrylamide intake reported for this specific population segment is greater than that reported for the whole population. This is the case of countries such as the Netherlands (Konings et al., 2003), Poland (Gielecinska, Mojska, & Szponar, 2007) and Germany (Mosbach-Schulz, Seiffert, & Sommerfeld, 2003) (Fig. 2). As documented by Dybing et al. (2005), the higher acrylamide intake of this population may be due to a combination of teens' higher caloric intake relative to body weight, as well as a higher consumption of certain acrylamide-rich foods, such as salty snacks.

The enKid study, developed by Serra-Majem and Aranceta (2002) in the Spanish adolescent population, recorded a consumption of 3.40 servings/week of salty snacks and 9.06 servings/week of bakery and biscuits for Spanish males aged 10–13 years. However, the participants in the present trial, following the recommendations, decreased their snacking. Only six of the subjects declared having eaten a salty snack during the experimental period and only a few, any type of biscuits, which are precisely among the foods considered as the greatest contributors to acrylamide intake. To come closer to the real situation of salty snacks and bakery products consumption established by Serra-Majem and Aranceta (2002), the following empirical calculation can be performed: if the salty snacks consumed are considered to be similar to potato crisps, that would mean an additional input of 0.130 μg/kg bw/day to the acrylamide daily exposure. Moreover, if the value of 21.3 μg acrylamide per serving used for the biscuits (Table 2) is applied to the data of 9.06 servings/week of bakery and biscuits, a second input of 0.493 μg/kg bw/day would be added. Thus, it can be speculated that, based on the current food habits of the Spanish adolescents, the daily acrylamide exposure could reach 1.15 μg/kg bw/day, which is twice that estimated in the present study, where the contribution of snacking was certainly little.

4. Conclusions

Although the above study has some limitations, primarily related to the inability to accurately repeat a culinary preparation, due to the variability of recipes, raw materials and manufacturers, it has been shown that the consumption of a balanced, traditional diet, with less snacking and fast food, significantly reduced the acrylamide daily exposure in our experimental conditions. The traditional diet contains less harmful levels of acrylamide according to health recommendations, and only when snacking and fast food are added, at the expense of the traditional diet consumption, do acrylamide levels raise considerably. Besides the usual beneficial effects on health, this is an additional argument for the preservation of the traditional diet versus the new dietary habits presented by young people.

Acknowledgements

This research was supported by a postdoctoral position and a project from Consejeria de Innovación, Ciencia y Empresa (Junta de Andalucia), as well as being partly funded by Consejeria Educacion y Ciencia (CAM) under the project (ANALISYC-II Program, S2009/AGR-1464). The authors would like to thank Miss Arribas-Lorenzo for practical assistance.

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