Journal of Food and Nutrition Research, 2017, Vol. 5, No. 12, 894-899
Available online at http://pubs.sciepub.com/jfnr/5/12/3

DOI:10.12691/jfnr-5-12-3

Assessment of the Dietary Intake of Acrylamide by Young Adults in Mexico

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Abstract The aims of this work were to estimate the content of acrylamide (AA) in food consumed by young people in Mexico and calculate its intake in this population sector. Fifteen samples of heat-processed commercial foods, widely consumed in Mexico obtained from supermarkets and local convenience stores, and analyzed in triplicate to determine their concentration of acrylamide (µg/kg of product) by HPLC-UV and later assess the intake in young adults. Significantly high contents of acrylamide were found in most foods evaluated; the highest levels were found in fried products (5,914 µg/kg of product in potato chips). Based on food and portions that subjects reported, an intake of Acrylamide of 0.68 µg/kg bw/day was estimated. The estimated daily intake could be at levels above the limit established to avoid the risk of neurotoxicity. This research provides relevant information for public health experts, policy makers and consumers in general.

Keywords: *Acrylamide, food-intake, health, México*

Cite This Article: M.G. SÁNCHEZ-OTERO, C.N. MÉNDEZ-SANTIAGO, F. LUNA-VÁZQUEZ, I. SOTO-RODRÍGUEZ, H.S. GARCÍA, and J.C. SERRANO-NIÑO, “Assessment of the Dietary Intake of Acrylamide by Young Adults in Mexico.” *Journal of Food and Nutrition Research*, vol. 5, no. 12 (2017): 894-899. doi: 10.12691/jfnr-5-12-3.

1. Introduction

Acrylamide attracted great public and scientific attention when the World Health Organization (WHO) published acrylamide concentrations in several foods in 2002 (WHO, 2002), as a result of reports from the Swedish National Food Authority and researcher of Stockholm University. In which it reports the presence of a carcinogen in experimental animals identified as acrylamide. Acrylamide (CH2=CH-CO-NH2; 2-propenamide; CAS Reg. No. 79-06-1AA) classified as probable human carcinogen in 1994 [2] is an organic compound that is widely used since the early 50's in the production of polyacrylamides used in the synthesis and production of co-polymers, colorants, fibers, textiles and additives for water treatment, also to prepare polyacrylamide gels for electrophoresis in biological laboratories and other applications [3,4]. Acrylamide is widely distributed in the diet of populations without been intentionally added to foods. The main mechanism for the formation of acrylamide in foods is through the Maillard reaction between reducing sugars (such as glucose or fructose) and the amino terminal of asparagine, during heat processing (T>120°C, 248°F); this is more pronounced in cereal products and potatoes [5].

Thermally processed products are consumed in large proportions by the general population, since thermal treatments can increase the range of color, flavor and aroma, also favor the development of desired sensory characteristics, thus offering more palatable products to consumers. Thermal processing extends the shelf life of food products, since microbial population is reduced or inactivated and moisture may be diminished. However, heating also favors chemical reactions that could promote the reduction in the content of beneficial compounds, or even in the formation of potentially toxicants as acrylamide [6,7].

Acrylamide has a double bond which favors its reactivity towards nucleophiles such as sulfhydryl, amino and carboxyl groups, all of them critical components in biologically important molecules such as nucleic acids and proteins, particularly the end sulfhydryl group (-SH) of cysteine, glutathione, free amino groups of amino acids, and N-terminal in proteins [3,4]. The main route of synthesis of acrylamide in foods is through the Maillard reaction between amino acids, particularly asparagine, and reducing sugars. However, it has been established that asparagine can undergo deamination and decarboxylation reactions during heating, thus leading to the formation of acrylamide. Additionally, the presence of compounds having carbonyl groups, as some alkadienes from aldehydes or lipid oxidation, and some intermediates of the Maillard reaction have been associated with a higher concentration of acrylamide in foods [6,8].

The consequences and mechanisms of acrylamide affinity to essential biomolecules for the maintenance of life have been widely studied since the early 2000's. Chronic and acute exposure to AA causes neurotoxicity effects in humans and rodents; it has been reported that single oral doses as high as four or five orders of magnitude (100,000 µg/kg bw/day), greater than the estimated daily intake of acrylamide (1-10 µg/kg bw/day) from food, have implications in genotoxicity, carcinogenicity, reproductive toxicity and development in rodents [6]. Male rodents subjected to acrylamide doses greater than 7 mg/kg bw/day showed a reduction in fertility and adverse effects on the morphology and counts of sperm cells; no adverse effects on fertility were observed in females [9]. In the past decade the correlation between acrylamide exposure in humans with neuropathy at both the Purkinje cells of the cerebellum, hippocampus and thalamus has been described, comprising the damage and degradation in distal axons and nerve terminals in the peripheral nervous system, which cause reduction of cognitive functions [3,10]. A recent proteomic study indicates that alkanes double bonded at the second carbon, including acrylamide, can cause toxicity by formation of irreversible adducts, that inactivate proteins after the reaction of acrylamide with nucleophilic groups. At least 100 different proteins of midbrain dopaminergic neuron cells in rat, formed adducts with acrylamide, representing 0.7% of the total cell proteome. These proteins are involved in metabolism, energy production and protein translation; all of these functions are necessary for cell homeostasis. Similarly, the analysis conducted by the authors, also indicated a connection between proteins that form adducts with acrylamide and those associated with a chronic degenerative disease like diabetes or atherosclerosis [4]. As for prenatal exposure, acrylamide toxicity has only been proven in the developing nervous system of rodents [11].

Although extensive cohort and case studies have been conducted in the United States and European countries, investigators have been unable to establish a direct correlation between acrylamide intake and the incidence/prevalence of various cancers; so that it was only possible to test the carcinogenicity of acrylamide in various organs of experimental animals [12,13]. Human exposure to various potentially carcinogenic compounds and factors in humans makes it difficult to demonstrate the correlation between acrylamide exposure and cancer incidence. Therefore, molecular studies for determining the concentration of acrylamide-DNA adducts that favor the development of this disease may prove to be conclusive [10,13]. Based on the above, the JECFA (JOINT FAO/WHO Expert Committee of Food Additives) (2011) and the EFSA considered acrylamide as a probable carcinogen [9,12]. The health risks for the general population are currently established within the limits of 1.0-4.0 µg/kg bw/day of oral exposure for moderate and high consumption respectively (i.e. 70-280 µg/day for a 70 kg individual). The safe limit to avoid neurotoxicity risks is considered below 200 µg/day. However, it has been reported that even consumptions below 300 total µg/day is not sufficient to protect against probable carcinogenic effects [8,13]. In countries like Canada, or China, daily acrylamide intake varies from 0.17 to 1.17 mg/kg bw/day, and this concentration depends on dietary patterns of each population [14,15]. The Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA, 2015), recently described data of the occurrence of acrylamide in foods based in ca. 7,500 results reported by 24 countries that combined demonstrate the exposure of the European population to this compound. Infants and children were the most exposed groups. Chronic dietary exposure of adolescents, adults, elderly and very elderly was estimated to be between 0.3 and 0.9 µg/kg bw/day and the 95th percentile between 0.6 and 2.0 µg/kg bw/per day. In Mexico, there are no official databases or tables that provide data to the general population in relation to the daily intake of acrylamide, even though the dietary patterns of the Mexican population generally contains elevated proportions of carbohydrates and/or use cooking techniques such as frying, roasting and baking [16,17]. Consequently, there is no legislation controlling concentration of acrylamide in thermally processed foods [16].

Acrylamide is recognized as a health risk due to its proven neurotoxic and probable carcinogenic effects; therefore, it is necessary to conduct survey studies where the concentration of acrylamide can be determined and used construct a reliable database to support bills so legislation in this regard can be established. The aim of this study was to determine the concentration of acrylamide in some thermally processed foods consumed in Mexico and make a first estimate of the dietary intake of the average Mexican population.

2. Materials and Methods

2.1. Sample Selection

Samples of various commercial starchy foodstuffs widely distributed in Mexico were selected randomly in triplicate in local supermarkets, fast-food restaurants and convenience stores in the city of Veracruz, state of Veracruz, Mexico, and were grouped in fifteen categories, to which acrylamide content was determined. These data are presented in Table 1. The samples comprised bread, crackers, French fries, roasted coffee, breakfast cereals, tortilla products, instant noodles, nuggets, potato chips and popcorn of different brands. The choice of food items was based on what was reported on the occurrence of acrylamide in foodstuffs reported by FAO/WHO and the Swedish National Food Administration [20].

2.2. Quantification of Acrylamide in Selected Foods

The samples were ground and homogenized with a food processor (Braun™). Briefly, 1.0 g of each sample was weighed and 10 mL of 0.1% (v/v) formic acid was added; the sample was homogenized using a magnetic stirrer (250 rpm, 20 min). Then, samples were centrifuged at 10,000 rpm (30 min, 4°C), and the supernatant was cleaned by Solid Phase Extraction columns (SPE C_{18} Max, Grace Pure™). These columns were conditioned with 1mL of methanol, followed by 1 mL of deionized water, then, 1 mL of sample was passed drop wise through the column in approximately 30 seconds; samples were eluted with 1 mL of deionized water and these fractions were collected and filtered through a 0.45μm syringe filter for further analysis by HPLC. The percent recovery of SPE columns was calculated by applying 1mL of acrylamide standard solution and its concentration was determined. The percent recovery value was used to adjust the acrylamide concentration of each sample. Samples of acrylamide extracted were analyzed using a HPLC system (Waters™, Milford, MA) with a C18 column reverse phase ODS Spheri-5 (220 mm x 4.6 mm, Supelco), a mobile phase of 0.1% formic acid; a UV detector (Waters 2487) adjusted 195 nm; a flow rate of 1 mL/min and an injection volume of 10 μL (EPA Method 8316). A calibration curve was constructed using the range of 0.5-1 μg acrylamide/mL to verify the linearity of the UV detector and different concentrations of the acrylamide standard were injected for 10 different days in order to evaluate the precision of the method. All samples were analyzed in triplicate. Values are presented as means ± standard deviations

2.3. Estimated Dietary Intake of Acrylamide Based in Food Consumption Data

The population sample consisted of volunteer subjects selected randomly. The sample size was estimated employing Eq. (1), with a confidence interval of 95 %.

$$n = \frac{N * Z^2 * p(1-p)}{N - 1 * e^2 + Z^2 * p(1-p)} \quad (1)$$

Where:
n = sample size
N = population size
Z = standard deviation
e = standard error
P = proportion

On the other hand, a record of three days consumption including Sundays was used to estimate the dietary intake of acrylamide. Subjects recorded the weights of foods consumed using household measurements (spoonful or cup). Consumption of individual food types and the content of acrylamide ingested (μg/kg bw/day) were estimated. Mann Whitney U test was used for statistical analysis. A Kolmogorov Smirnov test was used to analyze the population distribution.

3. Results

AA was analyzed employing a HPLC-UV system. To assess the linearity of the UV detector a calibration curve was constructed with concentrations of 0.5-1 μg/mL of acrylamide, and a linear coefficient of 0.9955 was calculated. Additionally, 10 different injections of an acrylamide standard (1 μg/mL) were made in 10 different days to evaluate the precision of the method employed, whereby a variation coefficient of 4.71 % was calculated. Quantification and detection limits were established for this method as 0.0622 and 0.0182 μg/mL, respectively. Finally, a spike of acrylamide with a concentration of 1 μg/mL was made to a food sample in order to confirm that the peak identified corresponded to acrylamide.

Table 1. Acrylamide (μg/kg) in various foodstuffs produced in México

	ACRYLAMIDE CONTENT (μg/Kg)
FOOD CATEGORY	Mean
1. Baguette bread	302 ± 4
2. Chocolate chip cookies	304 ± 0.53
3. Cracker biscuits	308 ± 12
4. White toast bread	320 ± 3
5. Instant noodles	331 ± 3
6. Roasted peanuts	347 ± 13
7. Fried chicken nuggets	364 ± 8
8. Baked tortilla	373 ± 22
9. Corn breakfast cereal	498 ± 19
10. Microwave popcorn	607 ± 36
11. Canned vegetables	957 ± 47
12. Medium toast ground coffee	1877 ± 71
13. Fried green plantain	1923 ± 32
14. French fries	3267 ± 142
15. Potato crisps	5802 ± 306

The AA content of the samples evaluated in present work are shown in Table 1. The samples analyzed consisted of foods subjected to different heat treatments: frying, baking, roasting and sterilization in the case of canned products and porridge for baby. The higher content of AA was found in fried foods such as potato chips, French fries and crushed plantain (5,802, 3,267 and 1,923 μg/kg of product, respectively). AA content was significantly high in products subjected to roasting; within these, the highest levels were found in medium roasted coffee. It is known that there are factors affecting acrylamide formation during the roasting process including roasting time, temperature and the variety of coffee; all can produce variations in the content of acrylamide. In products subjected to cooking and/or pasteurization, the highest concentration of AA was found in canned vegetables, which consisted of potato and carrot mixtures and instant noodles (957 and 331 μg/kg of product). With respect to baked goods, the highest content was found in corn products, baked tortillas, corn breakfast cereal and popcorn; in these products acrylamide concentrations were 373, 498 and 607 μg/kg of product, respectively. In baked goods, bread type baguette, cracker biscuits and chocolate chip cookies had lower levels of acrylamide (302, 308 and 304 μg/kg of product, respectively). In the case of baked bread, greater acrylamide concentrations have been reported, and it occurs in the crust of bread and, due to the high amount of asparagine in wheat endosperm, starch may harbor the highest amounts of acrylamide are found in baked bread.

3.1. Estimated Dietary Intake of Acrylamide Based in Food Consumption Data

In order to make a preliminary assessment of acrylamide intake only attributed to these products, surveys were conducted on 275 subjects; 36.5 % of the population sample (100) were men and 63.5 % (174) were women, the mean age was 22 ± 2 years. On the other hand, an intake of 0.68 µg/kg bw/day was estimated based on food and portions that subjects reported, as it can be noted in Table 2. The daily intake of Acrylamide was estimated according to gender, and the data are summarized in Table 3. Data distribution was not normal (Kolmogorov Smirnov test, $p < 0.0001$). The results indicated that males had lower exposure to acrylamide than females; however, there was no significant differences in acrylamide consumption between women and men (µg / 3 days) p=0.3500 U = 8109. There are no significant differences in acrylamide consumption between women and men (µg / kg / weight) p=0.8912 U = 8613 and this could be attributed to body weight, which was lower in females than in males (68.7 and 72.0 kg, respectively) and acrylamide distribution was greater in males.

Table 2. Consumption of acrylamide in surveyed population (n=275)

Food	Acrylamide µg/kg product	% Consumers
Baguette bread	5.94±9.05	54.01
Chocolate chip cookies	3.60±13.22	34.31
Cracker biscuits	0.54±2.79	9.49
White toast bread	0.31±1.39	8.03
Instant noodles	0.14±1.72	1.09
Roasted peanuts	0.20±1.63	2.92
Fried Chicken nuggets	0.25±4.13	0.36
Baked tortilla	0.82±3.55	15.69
Corn breakfast cereal	2.18±7.78	19.71
Microwave popcorn	0.92±4.3	6.57
Canned vegetables	0.24±4.05	0.36
Medium toast ground coffee	2.75±6.95	32.85
Fried green plantain	4.09±20.51	4.38
French fries	9.0±35.32	9.12
Potato crisps	14.88±64.89	9.85

Table 3. Acrylamide consumption according to gender

	Mean Weight (kg)	Acrylamide intake (µg/kg bw/day)
Men	72.06 ±0.52	0.65 ±1.07
Women	68.73 ±0.74	0.70 ±1.28

4. Discussion

The higher content of acrylamide was found in fried foods: potatoes chips, French fries and green plantain chips. These results should be considered of concern since only in 2000 the demand from the food industry in México was of more than 287 tons of fresh potatoes only for the production of potato chips [18]. Additionally, the market for sweet and savory snacks in Mexico is still growing, so as the total sales of this sector in 2002 reached US $ 1,885 million and by the end of 2007, this sector posted total sales of US $ 2,597 million, representing an increase of 37.8% and an increase of 6.6% between the years 2006 and 2007. According to the Euromonitor, in 2012 the market was US $ 3.21 billion [19]. High levels of acrylamide in toasted products like medium roast coffee were found in this study. Our results contrast with those provided by European countries in the recent Draft Scientific Opinion on Acrylamide in Food, in which it is stated that one of the products with the highest acrylamide contents is coffee or coffee substitutes with an average level of 578 µg/kg of product [12]. Despite that the acrylamide content in coffee is affected by variables such as roasting time, temperature and coffee bean variety, our results are of concern since Southern Mexico is one of the regions where coffee consumption is higher than other areas. Coffee consumption has increased 35 % from 2005 to 2010. In the later year, the per capita consumption was 1.35 kg of green coffee and it was expected to increase to 1.85 kg by 2015 [19]. In products subjected to cooking and/or pasteurization, the highest concentration of acrylamide was found in canned vegetables that consisted in a mixture of potatoes and carrots; however, instant soups also contained measurable amounts of acrylamide. Instant soups are a source of alarm, since these products are widely consumed by the Mexican population; where more than 514 million servings per year were reported in 2006 [20].

In the baked foods that we analyzed, the highest level was found in corn products such as baked tortilla chips, cornflakes for breakfast and popcorn. In México as in other Latin America countries, corn products are the most important sources of protein, calcium and energy. The annual consumption of tortillas in México in 2006 amounted 13.6 million tons (SIAP). Tortilla is one of the most popular products in Mexico; in their preparation, corn is subjected to a nixtamalization process. It is a process in which the grain is soaked and cooked in an alkaline (lime) solution, and hulled, then the grain is milled to form dough, which is cooked to prepare tortillas [17]. This process also breaks down the proteins in the grain, increases the availability of amino acids and therefore, the chance to react with the sugars to produce acrylamide during the cooking time, or when the tortilla is being subjected to extra cooking process to prepare baked or fried chips [21]. A more recent report indicates that frying tortilla in soybean oil at 180°C for 3.15 min produced up to 802 µg/kg of AA [22]. We found in baked tortillas AA in concentrations that were lower than those reported by the later authors, who found that baking tortilla for four minutes produced an AA concentration of 587 µg/kg product. Baking parameters such as time and temperature could affect these values.

According to information provided by the Federal Office of Consumer of México [23], households in urban Mexican population where there are children who eat cereal like this, 50% of children consume two to three times a week, and 37% ingest it daily. This represents another significant source of acrylamide in infants in addition to snacks. It was estimated in 2006 that the corn demand for breakfast cereals and snacks was 500,000 tons per year. Based on this, the acrylamide content in popcorn, which is a very popular product in many countries, was also significant.

The variation in acrylamide levels in other baked products e.g. baguette bread, cracker biscuits and chocolate chip biscuits, can be explained by differences in the parameters of cooking and processing, the type and quality of the raw materials and the high variability in the formulations, and even storage conditions [24,25].

4.1. Estimated Dietary Intake of Acrylamide Based in Food Consumption Data

Acrylamide intake has been estimated by various studies Freisling *et al.* [26], discussed that the assessment of Acrylamide intake is particularly difficult because AA levels depend largely on the nature and extent of heat treatment and preparation methods. Mean acrylamide intake was 45.84 µg/day and 0.68 µg/kg bw/day based on food and portions that subjects reported, as it can be noted in Table 2. Despite the fact that many other foodstuffs analyzed in this work are not included in the population surveyed, or the concentration of acrylamide in many others that population consumes is not reported in México, any additional minor contribution of a few mg/day of acrylamide can not be underestimated. Our results are in the same order of magnitude than the report by Sirot et al. [27], in their study on French population; they assessed the exposure of general adult and children populations by combining analytical results with national consumption data. Sirot et al. reported a mean acrylamide exposure of 0.43±0.33-µg/kg bw/day for adults and 0.69±0.58-µg/kg bw/day for children. Authors established that these values indicated a health concern, since the calculated margins of exposure, based on benchmark dose limits defined for carcinogenic effects remain very low, especially for young children. In 2003 in Sweden, an average dietary intake of acrylamide in Sweden for an adult person of approximately 31 µg/day was reported. The authors proposed that this dietary intake of acrylamide could be associated with potential health risks [28]. Dybing et al. [29], reported a higher intake of acrylamide in boys from Norway and Belgium; these boys had a lower intake compared to girls in Brazil. In relation to age, the available data suggests that it is likely that younger people have higher AA intakes compared to older people when expressed in kg of body weight. In this work we found that females had a higher AA intake than males, but without significant differences. Despite of this, higher intake in females could be caused by corporal weight since female weight was lower than males (68.7 and 72.0 kg, respectively) and thus acrylamide distribution was wider in males. These results are different to the data presented by Matthys et al. [30], who found that acrylamide intake was higher in boys than in girls and they attribute this to the fact that boys have an overall higher intake of food than girls. Regarding the body weight, it has been reported that in the case of children and young people that have a lower average body weight, the exposure could be even larger for those groups of individuals compared to adults with a higher body weight.

5. Conclusions

Evaluation of the acrylamide content in various foodstuffs in México, especially those associated with high consumption, should generate interest in all concerned sectors: food industry, health management and government regulatory agencies. Studies aimed to evaluate the effect of diverse cooking variables in home condition on acrylamide production in various foods are currently under way. Data disclosure will be necessary so that people responsible for preparing foods in Mexican households and food services, such as industrial canteens, restaurants, may increase their awareness. There is no legislation or Federal instance in Mexico that monitors or regulates the acrylamide content in heat-treated foods, and could press the domestic food industry to take steps to lessen its presence. Moreover, in Mexico the levels of acrylamide in different food groups that contribute significantly to the dietary intake of the population are not known. General studies in this topic are still needed.

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