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Original Research Article

# Carcinogenic and neurotoxic risks of acrylamide and heavy metals from potato and corn chips consumed by the Lebanese population

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ABSTRACT

The present study aims to quantify acrylamide and metals in potato and corn chips and to determine their carcinogenic and neurotoxic risks. Gas chromatography mass spectrometry analysis revealed that the average acrylamide level in potato and corn chips (1756 μg/kg) was 3500-fold higher than the permissible limit for acrylamide in drinking water (0.5 μg/kg). Potato-based chips and baked chips were found to contain 23% and 18% more acrylamide than corn-based chips and fried chips, respectively. The daily consumption of acrylamide from potato and corn chips was found to be 7–40-fold higher than the risk intake for carcinogenesis set by World Health Organization (WHO) but was below the neurotoxic risk threshold. Energy dispersive X-ray fluorescence and thermal atomic absorption analysis revealed that the mean concentrations of zinc, lead and cadmium in corn chips were approximately 1.5-, 1.7- and 2.4-fold higher than the permissible limits set by Food and Agriculture Organization/WHO, respectively. However, the daily intake of these metals was lower than the oral reference dose and the upper tolerable daily intake set by the US Food and Drug Administration. The cancer risk for the Lebanese population from acrylamide exposure estimations appears to be significant, highlighting the need to conduct further epidemiological studies and ensure monitoring of acrylamide levels in food products.



## 1. Introduction

Acrylamide has been used in the chemical industry for the production of polyacrylamides which have extensive uses in the paper and textile industries, wastewater treatment facilities, tunnels and sewers construction, electrophoresis gels and even cosmetics (Shipp et al., 2006). On the other hand, acrylamide is neurotoxic to both humans and laboratory animals due to its damaging effects on the central and peripheral nervous systems (Hagmar et al., 2001). Animal studies have also shown that acrylamide causes an increase in the incidence of tumors in several glands, including thyroid, mammary, pituitary and other (Friedman et al., 1995). In addition, chronic intake of acrylamide-containing food has been associated with an increased risk for atherosclerosis (Naruszewicz et al., 2009). Consequently, acrylamide has been declared as "a probable human carcinogen" by the International Agency for Research on Cancer (IARC, 1994). The carcinogenic potential of acrylamide includes gene mutations and

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DNA damage in vitro and in vivo, in addition to being a germ cell mutagen (FAO/WHO, 2002).

Acrylamide is formed through the Maillard reaction from free asparagine and reducing sugars when they are heated at high temperatures (>120 °C) (Mottram et al., 2002). The Swedish National Food Administration reported the presence of elevated amounts of acrylamide in several carbohydrate-rich heat-processed foods, primarily potato and cereal products, which contain considerable amounts of precursors for the Maillard reaction (Lineback et al., 2012). The concern over the presence of acrylamide and its toxicity in regularly consumed food products has raised global interest to assess the possible risks to human health.

The study of heavy metals in the human diet is also critical because of these elements' dual effect as either essential or toxic to the human body (Korfali et al., 2013). Zinc, copper, chromium, cobalt, manganese and iron are essential nutrients and have therapeutic applications, as long as they do not exceed recommended daily intake (WHO, 1996b). In fact, the human body does not have a good mechanism for the elimination of heavy metals; thus chronic low-level intake can lead to damaging effects (Zurera et al., 1989).

Potato and corn products constitute a major component of the human diet in many cultures, and hence the risk of contamination would have a significant impact on human health. The release of hazardous pollutants in the environment is one of the major causes of food-supply contamination. Heavy metal contamination can occur during growing, handling or processing of crops (López-López et al., 2008). For example, contamination in vegetables may result from the soil, irrigation system, fertilizers and pesticides (Zhu et al., 2011). Potato and corn products, such as chips, are among the most highly consumed social snacks worldwide, and consequently excessive consumption of such products can be associated with high levels of heavy metal intake (Somer, 1974). Worldwide, few studies have been conducted to measure heavy metal concentrations in potato and corn chips (Gopalani et al., 2007), and none has been carried out on products sold in the Lebanese market.

The present study aims to (i) determine the daily consumption of acrylamide and heavy metals from local and imported potato-based and corn-based chips among the Lebanese population and (ii) assess associated carcinogenic and neurotoxic hazards. To the best of our knowledge, this study is the first of its kind for the Middle East and the Arab world, and is in-line with WHO recommendations and calls for additional investigations of acrylamide and heavy metals in food in developing countries (WHO, 2005).

## 2. Materials and methods

### 2.1. Chemicals, reagents and instruments

Acrylamide (95%), *n*-hexane (95% v/v), propan-1-ol (99% v/v), acetonitrile (95% v/v), nitric acid (65–70% v/v), phosphoric acid (85% v/v) and hydrogen peroxide (30% v/v) were of analytical grade and purchased from Sigma–Aldrich Chimie GmbH, Taufkirchen, Germany. Ultrapure water (15 MΩ cm) was obtained by purifying distilled water with a Milli-Q™ PLUS system (EMD Millipore, Billerica, MA, USA). Spectroscopic analysis was carried out using a Hewlett Packard (California, Unites States) HP6890 Gas chromatography–Mass Spectrometer (GC–MS) equipped with NIST11 and Wiley9 MS databases. Statistical analyses were performed using the "Statistical Package for the Social Sciences (SPSS)" software 21.0 (SPSS, Chicago, IL, USA). Statistical significance was considered when $p < 0.05$.

### 2.2. Chemical analysis

Local and imported brands of chips were collected randomly from various locations across Lebanon (Byblos, Amchit, Tyre and Beirut), as duplicate samples, with different production dates; they were tested within a 2-month period. Samples ($n = 51$) were classified according to their nature: potato or corn, and baked or fried.

#### 2.2.1. Washing step

Each sample running in gas chromatography–mass spectrometry (GC–MS) was preceded by a washing step which consisted of a manual injection of methanol (1 μL). Helium was used as a carrier gas with splitless injection and a flow rate of 15.0 mL/min was applied. The temperature program was 4 min for solvent delay, 5.0 min at 75 °C, from 75 °C to 230 °C at 10 °C/min and held for 2.0 min.

#### 2.2.2. Acrylamide analysis

Acrylamide was extracted according to the method proposed by Biedermann and coworkers (Biedermann et al., 2002). Each chips sample (10 g) was ground, mixed with water (30 mL) and heated at 70 °C for 30 min. The homogenate (2.5 g) was mixed with propanol (10 mL) and centrifuged for 10 min at 2200 rpm and 20 °C. The supernatant (8 mL) was concentrated under reduced pressure before acetonitrile (2 mL) and hexane (5 mL) were added and the mixture sonicated for 3 min. The acetonitrile layer was re-extracted with hexane (5 mL), filtered using an SPE filter and analyzed via GCMS fitted with a fused silica HP5-MS 5% phenyl methyl siloxane cap column (30.0 m × 250.00 μm i.d., film thickness 0.25 μm) and directly coupled to the MS. The carrier gas was helium with splitless injection and the flow rate of 1.2 mL/min was applied. The temperature program was 15.0 min at 75 °C, from 75 °C to 230 °C at 10 °C/min and held for 45.0 min. An HP5973 mass selective detector was used in full scan electron ionization (EI) mode, and data were acquired over the range $m/z$ 50–500. Identification of acrylamide was achieved by comparing mass spectral data against the NIST11 and Wiley9 MS databases along with selective ion monitoring of the molecular ion at an $m/z$ of 71 (Supplementary Figs. S1 and S2). The concentration of acrylamide (μg/kg chips; Supplementary Fig. S3) was determined by comparing experimental results against a calibration curve obtained from standard acrylamide solutions prepared in the range of 50–500 mg/L. The limit of quantification (LOQ) was found to be 5.0 μg/L using the blank value method while the limit of detection (LOD) was found to be 0.5 μg/L. Acrylamide recovery was consistent with the reported average of 70–80% (Biedermann et al., 2002).

#### 2.2.3. Metal analysis

Samples were ground and homogenized into fine powder using a sieve shaker. The concentrations of Ca, K, Fe, Mn, Zn and Cu were determined using energy dispersive X-ray fluorescence (EDXRF) (Niton XL3 GOLLD hand held, Thermo Fisher Scientific) with up to 50 kV X-ray tube source and optimized silicon drift detector (SDD). Cd, Pb, Cr, Mo, Co, Ni and As were analyzed using the digestion procedure followed by thermal atomic absorption-graphite furnace ("Shimadzu" AA-6300) and background correction deuterium lamp (Supplementary Figs. S4–S9). The digestion procedure was carried out as follows: A solution of $H_2O_2$:$HNO_3$ (1:1 v/v, $v = 10$ mL) was added slowly to 1 g of chips, and the resulting mixture was stored at room temperature for 48 h, heated until it turned clear, sonicated for 5 min and then filtered using 0.45 μm pore-sized filter mesh. The solution (in a lidded plastic tube) was then diluted to 25 mL using deionized water and heated for 30 min at 60 °C in a water bath. After this step, phosphoric acid (170 μL) was added as a modifier and the tubes were shaken and stored at