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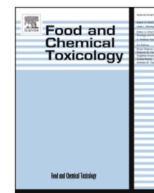

Determination of acrylamide levels in potato crisps and other snacks and exposure risk assessment through a Margin of Exposure approach

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ABSTRACT

Potato crisps, corn-based extruded snacks and other savoury snacks are very popular products especially among younger generations. These products could be a potential source of acrylamide (AA), a toxic compound which could develop during frying and baking processes. The purpose of this study was the assessment of the dietary intake to AA across six groups of consumers divided according to age through the consumption of potato crisps and other snacks, in order to eventually evaluate the margin of exposure (MOE) related to neurotoxic and carcinogenic critical endpoints. Different brands of potato crisps and other popular snacks were analyzed through a matrix solid-phase dispersion method followed by a bromination step and GC-MS quantification. The concentration of detected AA ranged from 21 to 3444 ng g^{-1} and the highest level occurred in potato crisps samples which showed a median value of 968 ng g^{-1}. The risk characterization through MOE assessment revealed that five out of six consumers groups showed higher exposure values associated with an augmented carcinogenic risk

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1. Introduction

Acrylamide (AA) (IUPAC name: 2-propenamide) is an organic compound, crystalline solid, colorless, odorless and highly soluble either in water or in other polar solvents and frequently used in industry in the manufacture of multi-purpose polymers as well as in molecular biology research (Erkekoğlu and Baydar, 2010; Riboldi et al., 2014). In 2000 the occurrence of AA in certain foods was hypothesized by Tareke et al. (2000) and later confirmed in 2002 (Svensson et al., 2003; Tareke et al., 2002, 2000). Besides, appreciable levels of AA can be found in tobacco smoke as well as in the environment, since its polymer (Polyacrylamide) is used in the manufacture of grouting agents, cosmetics, flocculants in water treatments, inks, building materials and many other products (Hagmar et al., 2017; Togola et al., 2015). Extensive research has been carried out on the formation mechanism of this compound in foodstuff: the main mechanistic pathway takes into account the Maillard reaction, whereby the AA is produced along with the flavour and the color of the foods, involving reducing sugars and amino acids as precursors (Elbashir et al., 2014; Notardonato et al., 2013). Thus, the occurrence of AA is especially common in fried and baked foods or at least in products whose composition reveals the presence of reducing sugars (mainly glucose and fructose) and free asparagine (Oracz et al., 2011; Russo et al., 2014; Sanny et al., 2012; Wyka et al., 2015). AA can also be formed through the elimination of ammonia from 3-aminopropionamide, a transient intermediate which develops during the decarboxylation of asparagine (Zyzak et al., 2003). Besides, some lines of evidence suggest that the formation of AA could also depend on nonspecific pathways involving the production of acrylic acid starting from other amino acids such as β-alanine (and related peptides like carnosine, which could release β-alanine via hydrolisis), cysteine and serine (Yaylayan et al., 2005; Yaylayan and Stadler, 2005). In any case, the production of AA during heat treatments is mainly due to the Maillard reaction and it is influenced by cooking time and temperature (Halford et al., 2012; Lim et al., 2014). Upon ingestion the AA is quickly absorbed from the gastro-intestinal tract and distributed to any organ and tissue in the human body (EFSA, 2015a; Fuhr et al., 2006). In view of its recognized toxicity, several studies about metabolism, toxicokinetics and mutagenicity of AA were carried out. The toxic effects exerted by AA are most likely to be due to glycidamide (GA), an epoxidic metabolite of AA which originates from the liver by means of the cytochrome P450 2E1 (Li et al., 2016; Pedreschi et al., 2014). A considerable amount of early and late

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in vitro and *in vivo* studies on rodents indicated that the AA and its metabolites may exhibit neurotoxicity, reproductive and developmental toxicity and genotoxicity (Attoff et al., 2016; Erkekoğlu and Baydar, 2010; Kadawathagedara et al., 2016; Katen et al., 2016; Zhao et al., 2016). Of greater concern though, are the carcinogenic effects widely documented in both sexes of rats and mice and which attracted worldwide attention since 1994 when the International Agency for Research on Cancer (IARC) classified this compound as potentially carcinogenic to humans (group 2A) (IARC, 1994; Manjanatha et al., 2015; Pelucchi et al., 2015; Xu et al., 2014). To date, several studies demonstrated that oral administration of AA in B6C3F1 mice and F344/N rats leads to a multi-organ carcinogenicity (Beland et al., 2013; EFSA, 2015a). Yet, as far as we know the existing literature lacks reliable and unambiguous epidemiological evidence on association between dietary intake of AA and risk of cancer (Virk-Baker et al., 2014). A number of authors focused on prospective cohort studies about the probable association between dietary AA intake and breast, endometrial, ovarian, renal and colorectal cancer, finding no clear relationship or coming to conflicting conclusions (Burley et al., 2010; Hogervorst et al., 2016; Je, 2015; Larsson et al., 2009; Mucci et al., 2004; Obón-Santacana et al., 2014). However, another crucial evidence is also that AA can pass through the human placenta (Annola et al., 2008) exerting fetal toxicity and influencing fetal growth and brain development (Allam et al., 2011; Duarte-Salles et al., 2013; Erdemli et al., 2016; Kadawathagedara et al., 2016). Even though the human exposure to AA may be related to multiple sources, the main contribution seems to be from diet, albeit among smokers the amount of smoked cigarettes per day should not be overlooked in a comprehensive assessment of the exposure to AA (Bjellaas et al., 2007; Mojska et al., 2012). As already mentioned, most of the available literature data that deals with occurrence of AA in foodstuff, found the highest concentrations of this compound to be occurring in baked and fried food. Thus, the human exposure to AA must be viewed in the light of the growing habit of consumption of potato crisps, cocktail snacks and other savoury snacks, especially among the younger generations (Tamanna and Mahmood, 2015; Wong et al., 2014). In light of these concerns, a reliable risk characterization of dietary exposure to AA has grown in importance in recent years. According to a scientific opinion of European Food Safety Authority (EFSA) an adequate assessment of neurotoxicity and carcinogenicity as critical endpoints of exposure to AA, should be based upon a benchmark-dose (BMD) approach, evaluated along with its lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) and related Margin of Exposure (MOE) (EFSA, 2015a).

Therefore, in consideration of the above, this study set out to investigate the occurrence of AA in most common potato crisps products and other savoury snacks, in order to provide a deterministic assessment of the dietary exposure to AA of different aged consumers through a MOE approach, making an important contribution to existing knowledge, underlining the importance of dietary choices, especially from a public health preventive perspective.

2. Materials and methods

2.1. Sampling

A total of 75 samples, each coming from different production batches, were bought at local stores of Campania region (Italy). Ten different brand were selected among the most common baked and fried snacks and consisted of: potato crisps, corn-based extruded snacks (corn curls, corn chips and cheese puffs) cocktail snacks and other flavoured croutons (cracker-like products and hereafter both identified as other savoury snacks) and ready-to-eat roasted peanuts. The samples were stored at 20 °C in their own package for a maximum of 7 days. Then the package was open and the sample was grinded into particles with size of about 0.5–1.0 mm, homogenized and stored at 20 °C before analysis that was performed within an hour after the homogenization.

2.2. Reagents and equipments

Acetonitrile, ethyl acetate, n-hexane (all GC grade) and sodium chloride (analytical grade) were purchased from Merck KGaA, (Darmstadt, Germany); AA (purity ≥ 99.8%) and the internal standard (IS) AA-$^{13}C_3$, potassium bromide, sodium thiosulfate pentahydrate (both analytical grade) and hydrobromic acid (48% w/w aq.) were supplied by Sigma Aldrich (St. Louis, MO, USA); preparative C18 (125 Å 55–105 μm) supplied by Waters (Milford, MA, USA); saturated bromine solution supplied by Titolchimica (Pontecchio Polesine, RO, Italy). Agilent 7890A GC-MS system coupled to an Agilent 5975C mass selective detector (MSD) (Agilent Technologies, Santa Clara, CA, USA) and equipped with a Restek Rxi®-XLB GC column (proprietary phase; length × I.D. 30 m × 0.25 mm; df: 0.25 μm) (Restek, Bellefonte, PA, USA).

2.3. Preparation of the analytical standards

A stock standard solution of 1 g L^{-1} was prepared dissolving 20 mg of AA in acetonitrile to a total volume of 20 mL and a working solution of 1 mg L^{-1} was obtained by appropriate dilution. The standards were processed in parallel to the samples and brominated, starting from aliquots of 1000, 500, 250, 125 and 62.5 μL of working solution (corresponding respectively to 1000, 500, 250, 125 and 62.5 ng of AA and equivalent to a range of 125–2000 ng g^{-1} in the samples). Likewise a working solution of 10 mg L^{-1} of IS was prepared.

2.4. Analytical method

The detection of AA in selected samples was performed by adapting the procedure used by Fernandes and Soares (2007) that applied a matrix solid-phase dispersion (MSPD) followed by bromination of aqueous extracts and thoroughly described below. 0.5 g of ground sample were added to 2 g of C18 sorbent phase and well mixed with a glass rod. Once the sample was well dispersed, the mix was packed into a polypropylene syringe with a polyethylene frit at the bottom and a second frit was placed onto the top of the packed mix. Then, the column was placed onto a vacuum manifold and sample was defatted with 20 mL of n-hexane at a flow rate of about 10 mL min^{-1} and then the column was dried by applying a vacuum for a few minutes; finally, AA was eluted adding 8 mL of water divided into two aliquots (4 + 4 mL) holding the flow for 5 min in order to permit a better extraction of the target compound. The extract was collected in an amber glass vial for the subsequent bromination reaction. The samples were analyzed in triplicate.

2.5. Bromination

To the aqueous extracts previously collected 1 g of calcinated KBr was added and the solution was acidified until pH 1–3 with HBr. Then, 2 mL of saturated bromine solution were added and samples were stored in ice bath for 2 h. Few drops of $Na_2S_2O_3$ 1 M solution were added to the sample until the yellow colour of the extracts disappeared (5–7 drops). Then, 4 g of NaCl were added to the extract and the brominated derivative was extracted firstly with 10 mL and then with further 5 mL of ethyl acetate:n-hexane mixture (4:1 v/v), pooling the organic layers and discarding the

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