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Determination of acrylamide in dried fruits and edible seeds using QuEChERS extraction and LC separation with MS detection

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ABSTRACT

Acrylamide is a carcinogenic and neurotoxic process contaminant that is generated from food components during heat treatment, while it is absent in raw foodstuffs. Its level in food arouses great concern. A method for acrylamide extraction and determination in dried fruits (dried prunes and raisins) and edible seeds (almonds, hazelnuts, peanuts, pine nuts, pistachios, and walnuts) using a QuEChERS-LC-ESI-MS-Triple Quadrupole approach was set up. Linearity, sensitivity, accuracy, and precision of the method were satisfactory.

Dried prunes and peanuts were the only samples appreciably contaminated, 14.7–124.3 and 10.0–42.9 μg/kg, respectively, as a consequence of the drying process. In fact, prunes are dried at 70–80 °C for a quite long time (24–36 h), while peanuts undergo a roasting process at 160–180 °C for 25–30 min.

The relative standard deviations, accuracy, LOD, and LOQ show that the method provides a reliable approach to acrylamide determination in different matrices.

1. Introduction

Acrylamide is a neo-formed contaminant (NFC), produced in food during manufacturing or home-cooking (Capuano & Fogliano, 2011). It is absent in raw foods and in raw materials used to make food, and it is produced and accumulated during thermal processing (FSA, 2012).

Global levels of dietary exposure to acrylamide indicate a human health concern (FAO/WHO, 2010).

Acrylamide was first classified as a potential carcinogen and neurotoxic to humans (Group 2A) based on its carcinogenicity in rodents in 1994 (IARC, 1994) and the suspicion was then endorsed in 2002 (WHO, 2002, SNFA, 2002, Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). For these reasons, acrylamide level has been strictly controlled by the authorities (EFSA, 2012) even if there is no existing legal limit for the concentration of this contaminant in foodstuffs. However, European Union fixed a maximum recommended level of 1000 μg kg^{-1} for potato chips (EU, 2013).

Acrylamide is formed primarily in carbohydrate-rich foods treated at high temperatures (i.e. >120 °C) (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2000; Tareke et al., 2002). The predominant chemistry involves the Maillard reaction, that occurs by a condensation of the amino group of the asparagine and the carbonyl group of reducing sugars during heating (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Stadler et al., 2004; Zyzak et al., 2003). Browned crispy crusts in foods like French fries, potato chips, coffee, crackers, pretzel-like snacks, cereals, and browned breads have the highest levels of acrylamide (EPA, 2010).

In addition, other reaction routes are involved in acrylamide formation in food. Acrolein (propenal), an unsaturated aldehyde, can be an acrylamide precursor and it can be produced from triglycerides by strong heat treatment (Umano & Shibamoto, 1987). Acrylic acid (Yasuhara, Tanaka, Hengel, & Shibamoto, 2003) and wheat gluten (Claus, Weisz, Schiebe, & Carle, 2006) are acrylamide precursor in other minor routes. Finally, 3-aminopropionamide is an effective precursor of acrylamide in

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the absence of further catalysts, such as carbonyls (Granvogl, Jezussek, Koehler, & Schieberle, 2004; Granvogl & Schieberle, 2006; Granvogl & Schieberle, 2007).

Acrylamide has been detected in some food products that are processed at temperatures in the 98–116 °C range, and in high moisture conditions [i.e., canned black olives (not cured oil) and prune juice] (Roach, Andrzejewski, Gay, Nortrup, & Musser, 2003). It is clear that other pathways of formation below 120 °C can yield acrylamide, and these are being further evaluated (JIFSAN, 2004).

Factors that are particularly important for the Maillard reaction are the starting reactants (Yaylayan & Stadler, 2005), i.e. kind of sugar and amino acid (protein), temperature, time and water activity. The presence of metal salts (pro-oxidants), and inhibitors, such as antioxidants and sulfite, may have an impact.

During analysis, acrylamide, a small hydrophilic molecule, is usually extracted with water but a polar organic molecule, such as the more volatile acetonitrile, is a suitable alternative (Tateo & Bononi, 2003). To extract acrylamide, different authors used an aqueous solution with high-concentration of NaCl to inhibit the formation of emulsions (Young, Jenkins, & Mallet, 2004), or water and 1-propanol on defatted samples (Biedermann, Biedermann-Brem, Noti, & Grob, 2002). Other authors introduced a deproteinating step (Gertz & Klostermann, 2002). However, all these sample manipulations can be bypassed by a QuEChERS approach (Anastassiades, Lehotay, & Štajnbaher, 2002; Mastovska & Lehotay, 2006). In comparison with a traditional strategy based on solid phase technique (SPE), the proposed method allows "one-pot" sample preparation thus limiting the amount of solvent used for the extraction. Remarkable time and money-per-sample savings are considerable, as well.

LC–MS/MS is the most used and authoritative method for acrylamide determination. Because its high sensitivity, LC–MS/MS avoids the derivatization step, that is time consuming and potentially unhealthy.

The aim of this study is to set up and apply the more efficient QuEChERS approach in order to extract and determine acrylamide in packed dried fruits (dried prunes and raisins) and some edible seeds (almonds, hazelnuts, peanuts, pine nuts, pistachios, walnuts).

2. Materials and methods

2.1. Sampling and grinding

Sixty-eight samples of packed dried fruits and edible seeds were purchased on the Italian market. In particular, dried prunes (13 samples) pitted (7 samples) and not pitted (6 samples), and raisins (7 samples) as dried fruits, and peeled almonds (2 samples), roasted and peeled hazelnuts (2 samples), roasted and salted pistachios (7 samples), pine nuts (7 samples) from Portugal (2 samples) and Italy (5 samples), walnuts (4 samples) with shell from USA (2 samples) and from Chile (2 samples), and roasted and salted peanuts (26 samples) with shell and from Israel (24 samples) and from Egypt (2 samples), as edible seeds.

A gross amount of 20 g of each sample was ground by Osterizer 12-speeds blender (Oster Manufacturing, Di Giovanni Srl, Bologna, Italy) for further elaboration.

2.2. Chemicals

All solvents and reagents were of analytical grade; acetonitrile (Chromasolv® Plus purity for LC–MS, Sigma-Aldrich®), petroleum ether, methanol (Chromasolv® Plus purity for LC–MS, Sigma-Aldrich®), and *n*-hexane were obtained from Fluka Sigma-Aldrich® S.r.l. (Milan, Italy). Anhydrous sodium sulphate was purchased from Carlo Erba Reagents S.p.A. (Rodano, Milan, Italy).

QuEChERS pouches containing $MgSO_4$ 4.0 g + NaCl 0.5 g were purchased from Agilent Technologies Italia S.p.A. (Milan, Italy). DisQuE QuEChERS tubes (1) containing $MgSO_4$ 8.0 g + CH_3COONa 2.0 g plus DisQuE QuEChERS tubes (2) containing PSA (Primary Secondary Amine – dSPE – technique) 25 mg and $MgSO_4$ 150 mg were purchased from Waters S.p.A. (Milan, Italy). Acrylamide used as external standard was purchased by Sigma-Aldrich® S.r.l. (Milan, Italy) with a purity of 99%.

2.3. QuEChERS protocol setup

Different protocols and materials for acrylamide extraction were tested. They included different clean-up steps (defatting by hexane or dispersive SPE clean-up), effect of injection solvent (acetonitrile), QuEChERS pouches (Agilent QuEChERS pouches containing $MgSO_4$ 4.0 g + NaCl 0.5 g or Waters QuEChERS tubes containing $MgSO_4$ 8.0 g + CH_3COONa 2.0 g), water volume (0, 2.5 mL, 5.0 mL), and sample weight (1.00 g, 2.50 g, 5.00 g).

2.3.1. Optimized extraction protocol for acrylamide

An aliquot of ground sample (2.50 g) was transferred into a 50-mL Falcon tube together with a ceramic homogenizer for QuEChERS. Then 5 mL of Milli-Q water and 10 mL of acetonitrile were added, and the tube was vigorously hand shaken for 1 min after the addition of each solvent. A prepared mix of QuEChERS pouch composed by $MgSO_4$ 4.0 g + NaCl 0.5 g was added and hand shaken for 1 min and for 3 min with a shaker (Unimax 2010, Heidolph Instruments Italia S.r.l., Milan, Italy) to separate acrylamide into the acetonitrile phase. The tubes were centrifuged for 3 min at 3000 rpm to separate the two layers. An aliquot of the upper layer (2 mL) was dried by a gentle nitrogen stream (20 min in a water bath at 40 °C). The sample was dissolved in 1 mL of Milli-Q water and filtered through a 0.22 μm PES membrane into a 2 mL vial that was loaded into autosampler chamber at controlled temperature, ready for analysis by LC–MS-MS. Each sample extraction was carried out in triple.

2.4. Acrylamide determination

The acrylamide determination was carried out by reverse phase liquid chromatography coupled with mass spectrometry system (Agilent Technologies, Waldbronn, Germany) consisting of a vacuum pump (Agilent 1200), a gas generator (API MM20 ZA; Peak Scientific Billerica, MA, USA), a degasser (Agilent 1200), a binary pump (Agilent 1200), an autosampler (Agilent 1200), a thermostated column compartment (Agilent 1200), and a mass spectrometer triple quadrupole (API 3200, AB Sciex Germany GmbH, Darmstadt, Germany). Samples (20 μL) were injected into a Gemini RP C_{18} column (Phenomenex, Torrance, CA, USA) (25 cm × 2 mm i.d. × 5 μm particle size × 110 Å pore size). The solvent system was 0.1% formic acid in water (99.5%, solvent A) and 0.1% formic acid in methanol (0.5%, solvent B) and the elution was carried out in isocratic mode (total run 7 min), with a flow rate of 0.25 mL/min at ambient temperature. The analysis was performed in double for all the samples.

The analyses were carried out in positive electrospray ionization mode (ESI+) and using the following conditions: curtain gas, 20.0 psi; collision activated dissociation, 7.0 (arbitrary units); ion spray, 5500.0 V; temperature, 700.0 °C; nebulizer gas, 70.0 psi; heater gas, 30.0 psi. The main MS parameters optimized for acrylamide determination were: declustering potential, 22.0 V; collision energy, 14.1 V; collision cell exit potential, 4.1 V; entrance potential, 6.0 V. The parent ion *m*/*z* was 72.1 and the qualifier ion