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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.

Ms. Ref. No.: FOODCHEM-D-15-03161R1

Dear Editor,

I am sending you a copy of the revised Ms. entitled: Determination of Acrylamide in Dried Fruits and Edible Seeds Using QuEChERS extraction and LC Separation with MS Detection.

All reviewers' comments were carefully considered and accepted in large part. English was revised, as well.

Yours sincerely,

Dr. Giuseppe Montevicchi

Ms. Ref. No.: FOODCHEM-D-15-03161R1

Reviewers' comments:

Reviewer #3

In my opinion, in the present form the manuscript titled 'Determination of Acrylamide in Dried Fruits and Edible Seeds Using QuEChERS extraction and LC Separation with MS Detection' described by Eleonora Laura De Paola, Giuseppe Montevicchi, Francesca Masino, Davide Garbini, Martino Barbanera, Andrea Antonelli can be recommended for publication in the Food Chemistry after minor revision.

My remarks and recommendations to the FOODCHEM-D-15-03161.R1 manuscript are as follows:

1. The matrix effect should be estimated as ratio of slopes (matrix/solvent).

QuEChERS sample preparation is a classical method to reduce matrix effect. Moreover, our study was carried out on different samples with very different matrices. For these reasons, we did not carry out a true “matrix effect study” but we considered the residual matrix effect (after QuEChERS purification) by spiking real sample technique.

For sake of precision and considering the reviewer observation, we rename 3.2.2 section “Accuracy evaluation”, only.

2. Number of experiments used to estimate the SD?

Done in Materials and methods (lines 137-138) and in Table 6. Each sample extraction was carried out in triple.

3. Neither, there is in the article a comparison of the characteristics of the method with other previously published, for example a comparison of the mLOQs to other methods previously published. Please, indicate clearly the advantages of the method proposed compared to other methods previously published.

The general advantage are now presented in the Introduction (lines 90-93) and Conclusions (lines 259-262), while the comparison with other methods previously published are reported in 3.2.1. (lines 204-205) and Conclusions (line 261-262).

Determination of Acrylamide in Dried Fruits and Edible Seeds Using QuEChERS extraction and LC Separation with MS Detection

Eleonora Laura De Paola, Giuseppe Montevicchi, Francesca Masino, Davide Garbini, Martino Barbanera, Andrea Antonelli

Highlights: > Acrylamide, a toxic process contaminant generated during heat treatment of food > QuEChERS approach was for the first time optimized for dried fruits and edible seeds > 68 samples of dried fruits and edible seeds purchased on Italian market were tested > Method linearity, sensitivity, matrix effect, accuracy, and precision were evaluated > Dried prunes and peanuts were the only samples contaminated with acrylamide

1 **Determination of Acrylamide in Dried Fruits and Edible Seeds Using QuEChERS extraction**
2 **and LC Separation with MS Detection**

3
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16

17

18 **Abstract**

19

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

26 Dried prunes and peanuts were the only samples appreciably contaminated, 14.7-124.3 and 10.0-
27 42.9 µg/kg, respectively, as a consequence of the drying process. In fact, prunes are dried at 70-80
28 °C for a quite long time (24-36 h), while peanuts undergo a roasting process at 160-180 °C for 25-
29 30 min.

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

32

33 _____

34

35 *Keywords:* Acrylamide; QuEChERS; LC-ESI-MS-Triple Quadrupole; Dried fruits; Dried prunes;
36 Peanuts.

37

38 *Chemical compounds studied in this article:*

39 Acrylamide (PubChem CID: 6579)

40

41 **1. Introduction**

42

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

46 Global levels of dietary exposure to acrylamide indicate a human health concern (FAO/WHO,
47 2010).

48 Acrylamide was first classified as a potential carcinogen and neurotoxic to humans (Group 2A)
49 based on its carcinogenicity in rodents in 1994 (IARC, 1994) and the suspicion was then endorsed
50 in 2002 (WHO, 2002; SNFA, 2002; Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). For
51 these reasons, acrylamide level has been strictly controlled by the authorities (EFSA, 2012) even if
52 there is no existing legal limit for the concentration of this contaminant in foodstuffs. However,
53 European Union fixed a maximum recommended level of $1000 \mu\text{g kg}^{-1}$ for potato chips (EU, 2013).
54 Acrylamide is formed primarily in carbohydrate-rich foods treated at high temperatures
55 (i.e. $> 120 \text{ }^\circ\text{C}$) (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2000; Tareke et al., 2002). The
56 predominant chemistry involves the Maillard reaction, that occurs by a condensation of the amino
57 group of the asparagine and the carbonyl group of reducing sugars during heating (Mottram,
58 Wedzicha, & Dodson, 2002; Stadler et al., 2002; Stadler et al., 2004; Zyzak et al., 2003). Browned
59 crispy crusts in foods like French fries, potato chips, coffee, crackers, pretzel-like snacks, cereals,
60 and browned breads have the highest levels of acrylamide (EPA, 2010).

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

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

68 Acrylamide has been detected in some food products that are processed at temperatures in the 98–
69 116 °C range, and in high moisture conditions [i.e., canned black olives (not cured oil) and prune
70 juice] (Roach, Andrzejewski, Gay, Nortrup, & Musser, 2003). It is clear that other pathways of
71 formation below 120 °C can yield acrylamide, and these are being further evaluated (JIFSAN,
72 2004).

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

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

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

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

93

94 **2. Materials and methods**

95

96 *2.1. Sampling and grinding*

97 Sixty-eight samples of packed dried fruits and edible seeds were purchased on the Italian market. In
98 particular, dried prunes (13 samples) pitted (7 samples) and not pitted (6 samples), and raisins (7
99 samples) as dried fruits, and peeled almonds (2 samples), roasted and peeled hazelnuts (2 samples),
100 roasted and salted pistachios (7 samples), pine nuts (7 samples) from Portugal (2 samples) and Italy
101 (5 samples), walnuts (4 samples) with shell from USA (2 samples) and from Chile (2 samples), and
102 roasted and salted peanuts (26 samples) with shell and from Israel (24 samples) and from Egypt (2
103 samples), as edible seeds.

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

106

107 *2.2. Chemicals*

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

112 QuEChERS pouches containing MgSO₄ 4.0 g + NaCl 0.5 g were purchased from Agilent
113 Technologies Italia S.p.A. (Milan, Italy). DisQuE QuEChERS tubes (1) containing
114 MgSO₄ 8.0 g + CH₃COONa 2.0 g plus DisQuE QuEChERS tubes (2) containing PSA (Primary
115 Secondary Amine – dSPE – technique) 25 mg and MgSO₄ 150 mg were purchased from Waters

116 S.p.A. (Milan, Italy). Acrylamide used as external standard was purchased by Sigma-Aldrich[®] S.r.l.
117 (Milan, Italy) with a purity of 99 %.

118

119 *2.3. QuEChERS protocol setup*

120 Different protocols and materials for acrylamide extraction were tested. They included different
121 clean-up steps (defatting by hexane or dispersive SPE clean-up), effect of injection solvent
122 (acetonitrile), QuEChERS pouches (Agilent QuEChERS pouches containing
123 MgSO₄ 4.0 g + NaCl 0.5 g or Waters QuEChERS tubes containing
124 MgSO₄ 8.0 g + CH₃COONa 2.0 g), water volume (0, 2.5 mL, 5.0 mL), and sample weight (1.00 g,
125 2.50 g, 5.00 g).

126

127 *2.3.1. Optimized extraction protocol for acrylamide*

128 An aliquot of ground sample (2.50 g) was transferred into a 50-mL Falcon tube together with a
129 ceramic homogenizer for QuEChERS. Then 5 mL of Milli-Q water and 10 mL of acetonitrile were
130 added, and the tube was vigorously hand shaken for 1 min after the addition of each solvent. A
131 prepared mix of QuEChERS pouch composed by MgSO₄ 4.0 g + NaCl 0.5 g was added and hand
132 shaken for 1 min and for 3 min with a shaker (Unimax 2010, Heidolph Instruments Italia S.r.l.,
133 Milan, Italy) to separate acrylamide into the acetonitrile phase. The tubes were centrifuged for
134 3 min at 3000 rpm to separate the two layers. An aliquot of the upper layer (2 mL) was dried by a
135 gentle nitrogen stream (20 min in a water bath at 40 °C). The sample was dissolved in 1 mL of
136 Milli-Q water and filtered through a 0.22 µm PES membrane into a 2 mL vial that was loaded into
137 autosampler chamber at controlled temperature, ready for analysis by LC-MS-MS. Each sample
138 extraction was carried out in triple.

139

140 *2.4. Acrylamide determination*

141 The acrylamide determination was carried out by reverse phase liquid chromatography coupled with
142 mass spectrometry system (Agilent Technologies, Waldbronn, Germany) consisting of a vacuum
143 pump (Agilent 1200), a gas generator (API MM20 ZA; Peak Scientific Billerica, MA, USA), a
144 degasser (Agilent 1200), a binary pump (Agilent 1200), an autosampler (Agilent 1200), a
145 thermostated column compartment (Agilent 1200), and a mass spectrometer triple quadrupole (API
146 3200, AB Sciex Germany GmbH, Darmstadt, Germany). Samples (20 μ L) were injected into a
147 Gemini RP C₁₈ column (Phenomenex, Torrance, CA, USA) (25 cm \times 2 mm i.d. \times 5 μ m particle
148 size \times 110 Å pore size). The solvent system was 0.1 % formic acid in water (99.5 %, solvent A) and
149 0.1 % formic acid in methanol (0.5 %, solvent B) and the elution was carried out in isocratic mode
150 (total run 7 min), with a flow rate of 0.25 mL/min at ambient temperature. The analysis was
151 performed in double for all the samples.

152 The analyses were carried out in positive electrospray ionization mode (ESI+) and using the
153 following conditions: curtain gas, 20.0 psi; collision activated dissociation, 7.0 (arbitrary units); ion
154 spray, 5500.0 V; temperature, 700.0 °C; nebulizer gas, 70.0 psi; heater gas, 30.0 psi. The main MS
155 parameters optimized for acrylamide determination were: declustering potential, 22.0 V; collision
156 energy, 14.1 V; collision cell exit potential, 4.1 V; entrance potential, 6.0 V. The parent ion m/z was
157 72.1 and the qualifier ion m/z was 54.9. These conditions were optimized by injecting acrylamide
158 solutions directly into the MS source.

159 Quantification was performed by external standard calibration and the chromatograms were
160 acquired and processed by Analyst software version 1.5 (AB Sciex).

161

162 **3. Results and discussion**

163

164 *3.1. Acrylamide extraction*

165 *3.1.1. Effect of defatting by hexane and dispersive SPE clean-up steps*

166 Mastovska et al. (2006) used hexane to defat samples and dSPE to clean up the extract. However, in
167 our samples the addition of 5 mL of hexane or the use of dSPE reduced acrylamide recovery at
168 about 20 %, and 36 %, respectively. For these reasons, these two steps were not included in the
169 protocol of acrylamide extraction.

170

171 *3.1.2. Effect of injection solvent on acrylamide determination*

172 Direct injection of the acetonitrile extract after QuEChERS altered the retention time during
173 chromatography. In addition, acetonitrile is a poor solvent for lipids that precipitated trapping part
174 of the acrylamide, thus lowering its recovery. To overcome this problem, two ways were carried
175 out: (i) a pre-dilution of the acetonitrile (250 μ L) with water (750 μ L), (ii) the complete acetonitrile
176 evaporation and the resolubilization of the sample with water.

177 Best results were achieved with the latter solution that was used throughout this study.

178

179 *3.1.3. Effect of two different QuEChERS pouches*

180 QuEChERS pouches with different composition (MgSO_4 4.0 g + NaCl 0.5 g and
181 MgSO_4 8.0 g + CH_3COONa 2.0 g) were compared.

182 QuEChERS with NaCl gave the best recoveries (Table 1), probably as a consequence of the reduced
183 emulsions (Young et al., 2004). On the contrary, QuEChERS with CH_3COONa had a negligible
184 effect on recoveries despite the observations of Eriksson & Karlsson (2006).

185

186 *3.1.4. Effect of water volume (MgSO_4 -NaCl pouches)*

187 To verify the effect of water volume on acrylamide recoveries, some tests on an acrylamide solution
188 in acetonitrile (250- μ g/kg) were carried out (Table 2). Different volumes of water were used: 0, 2.5,
189 and 5.0 mL. To meet a reasonable compromise between recovery and a good sample dispersion,
190 5.0 mL were used for further experiments.

191

192 *3.1.5. Effect of sample weight*

193 Comparison of the recoveries of different sample weights was carried out (Table 3). A 2.50-g
194 sample amount was a reasonable compromise between the highest recoveries and the necessity to
195 have a sample amount large enough to be representative.

196

197 *3.2. Method evaluation*

198 *3.2.1. Linearity and sensitivity determination*

199 Increasing concentrations of acrylamide, from 1 µg/kg to 500 µg/kg, were used to study the range of
200 linearity. As neither EFSA nor FSA suggested limits for dried fruits, we used those ones
201 recommended for biscuits, wafers, and crisp bread (EFSA 2012; FSA 2012).

202 The coefficient of determination R^2 was 0.999, showing a very good linearity.

203 Sensitivity of the method was determined with the limit of detection (LOD, 2.0 µg/kg) and the limit
204 of quantification (LOQ, 5.0 µg/kg) that were calculated by the calibration curve. These values were
205 lower in comparison with what found in different studies carried out with SPE extraction (Wenzl,
206 Karasek, Rosen, Hellenäs, Crews, Castle & Anklam, 2006; Bortolomeazzi, Munari, Anese &
207 Verardo, 2012).

208

209 *3.2.2. Accuracy evaluation (recoveries) and inter-laboratory essay*

210 One sample for each matrix was spiked with 250 µg/kg of acrylamide. The recoveries were
211 calculated for each sample (Table 4). For calculations of real samples, correction factors based on
212 the recoveries for each matrix were applied. Calibration was verified each day with three spiked
213 samples at different concentrations.

214 To test the adaptability of the method to extract acrylamide from other matrices, it was used a
215 European reference matrix (ERM), crisp bread (ERM-BD272) used in Food Analysis Performance
216 Assessment Scheme (FAPAS) for proficiency test 3024 (FAPAS, 2009; Koch, Bremser, Koeppen,
217 Siegel, Toepfer, & Nehls, 2009) to evaluate inter-laboratory variability. Even applying the poorest

218 recovery (61 %), the concentration of acrylamide was in the range of acceptability (Table 5). The
219 same test was then applied to rusks, as this matrix was the most similar to ERM (Koch et al., 2009).
220 Rusks were added with 250 µg/kg acrylamide and, even in this case, results were in the range of
221 acceptability (Table 5).

222

223 *3.2.3. Evaluation of precision: repeatability*

224 For the determination of the precision, four levels of contamination were considered: (i) a sample
225 not contaminated (pine nuts, acrylamide < LOD), (ii) a sample with a not quantifiable concentration
226 of acrylamide (pistachios, acrylamide < LOQ), (iii) a sample with a medium amount of acrylamide
227 (peanuts, 42.86 µg/kg), and (iv) a sample with a high concentration of acrylamide (dried prunes,
228 124.26 µg/kg). Each sample was extracted and injected three times. With acrylamide lower
229 concentrations (5 - 25 µg/kg) relative standard deviation (RSD) was 20 %, while with higher
230 acrylamide concentrations (26 - 124 µg/kg) RSD was 10 %.

231

232 *3.3. Results obtained on real samples*

233 Raisins, almonds, hazelnuts, pine nuts, and walnuts showed values lower than LOD, as well as
234 pistachios, except for one sample that was lower than LOQ. For this reason the results of these
235 samples are not shown.

236 These very low concentrations were expected because dried fruits and edible seeds are generally not
237 subjected to high temperature treatments. In literature, it was reported that acrylamide levels in
238 almonds were below 200 µg/kg if the roasting temperature was below 146 °C (Zhang, Huang, Xiao,
239 Seiber, & Mitchell, 2011). These data were recently confirmed by Schlörmann and coll. (2015).

240 Dried prunes and peanuts (Table 6) showed acrylamide content ranged from 14.74 µg/kg to 124.26
241 µg/kg, and from 6.16 µg/kg to 42.86 µg/kg (with two samples < LOQ and one < LOD),
242 respectively. The RSD were within 20 %, very good results if compared with 47 % found for
243 roasted kernel (Amrein, Lukac, Andres, Perren, Escher, & Amadò, 2005) and 60 % for dried prunes

244 (Amrein, Andres, Escher, & Amadò, 2007). The lower manipulation of the sample was likely the
245 reason of these good results.

246 Amrein and coll. (2007) detected from 730 to 1680 µg/kg of acrylamide in dried prunes. They also
247 heated again dried prunes at 120 °C for 40 min and they found an increase in acrylamide content
248 (up to 2240 µg/kg). In this case, time is the key factor for acrylamide formation. In fact, even if the
249 drying process of the prunes is carried out at quite low temperature (70-80 °C), the time of exposure
250 is long enough (24-36 h) to allow acrylamide formation. Moreover, the drying process (up to about
251 18 % moisture) enhances sugar and asparagine concentration (Amrein et al., 2007), thus fostering
252 acrylamide formation.

253 In the case of peanuts, the roasting temperature is the key factor for acrylamide accumulation. In
254 fact, temperatures are considerably higher (160-180 °C) for 25-30 min of roasting time.

255

256 **4. Conclusions**

257

258 The study describes a quick and easy method for acrylamide determination by QuEChERS
259 approach coupled to LC-ESI-MS-Triple Quadrupole technique with a “one-pot” sample
260 preparation. The method was applied for the first time to food matrices such as dried fruit (prunes
261 and raisins) and some edible seeds. Besides solvent saving, the proposed approach is more sensitive
262 and repeatable, with lower LOD and LOQ.

263 Results confirm that acrylamide was found with significant concentrations in dried prunes and
264 peanuts as a consequence of the thermal process they were submitted. In general, data agree with
265 those of literature. Other products have shown minimal acrylamide amounts, thus posing reduced
266 harm to humans.

267 For these reasons and for its flexibility to different products, this method is very promising for
268 acrylamide determination in other matrices, as well.

269

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271

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274

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Table 1.

Comparisons between two different QuEChERS compositions.

QuEChERS pouches	Sample weight (g)	Recoveries (%)	
		Almonds	Pistachios
MgSO ₄ - NaCl	1.00	60 ± 5	69 ± 6
	2.50	70 ± 2	73 ± 2
MgSO ₄ - CH ₃ COONa	1.00	54 ± 7	30 ± 8
	2.50	58 ± 7	58 ± 6

Table 2.

Composition of the trials and recoveries (%) found using different water contents to disperse the sample and dissolve acrylamide. The volume of acetonitrile was 10 mL and the final acrylamide concentration was 250 $\mu\text{g}/\text{kg}$.

Trials	H₂O volume (mL)	MgSO₄- NaCl	Recoveries (%)
1	No	No	40 \pm 8
2	No	Yes	83 \pm 6
3	2.5 mL	Yes	107 \pm 3
4	5.0 mL	Yes	117 \pm 4

Table 3.Effect of sample weight on recoveries (%) of acrylamide (250- μ g/kg spike).

Sample weight (g)	Dried Prunes	Raisins	Almonds	Pistachios
1.00	84 \pm 10	72 \pm 8	70 \pm 9	68 \pm 8
2.50	61 \pm 2	61 \pm 3	81 \pm 8	73 \pm 7
5.00	36 \pm 7	34 \pm 8	54 \pm 9	38 \pm 8

Table 4.

Recoveries (%) calculated for each matrix, spiked with 250 µg/kg of acrylamide.

Samples	Recoveries (%)
Almonds	70 ± 4
Hazelnuts	76 ± 3
Peanuts	77 ± 3
Pine nuts	63 ± 8
Pistachios	62 ± 7
Walnuts	82 ± 6
Dried prunes	61 ± 2
Raisins	61 ± 3

Table 5.

FAPAS proficiency test results. ^aThe range of acceptability expected by FAPAS included values in the range of Z score between -2 and 2. It was calculated that $Z = -2$ corresponded to 165 $\mu\text{g}/\text{kg}$ of acrylamide, while $Z = 2$ to 377 $\mu\text{g}/\text{kg}$, so that $Z = 0$ is 271 $\mu\text{g}/\text{kg}$. The standard deviation to apply (± 106) was corresponding to 39% RSD (FAPAS, 2009).

Sample	Recoveries (%) applied	Acrylamide content ($\mu\text{g}/\text{kg}$)	Range of acceptability^a ($\mu\text{g}/\text{kg}$) \pm standard deviation
Crisp bread (ERM-BD272)	61	167	271 ± 106
Rusks (spiked with 250 $\mu\text{g}/\text{kg}$ of acrylamide)	61	277	271 ± 106

Table 6.

Acrylamide content ($\mu\text{g}/\text{kg}$) in dried prunes (DP) and peanuts (PE). Each sample was triplicated.

LOD = limit of detection; LOQ = limit of quantification; SD = standard deviation.

	DP1	DP2	DP3	DP4	DP5	DP6	DP7	DP8	DP9	DP10	DP11	DP12	DP13
$\mu\text{g}/\text{kg}$	14.75	71.15	76.07	71.80	72.46	45.25	124.26	44.59	14.92	101.31	104.59	34.75	16.75
SD (\pm)	2.95	7.11	7.61	7.18	7.25	4.52	12.43	4.46	2.98	10.13	10.46	3.48	3.35
	PE1	PE2	PE3	PE4	PE5	PE6	PE7	PE8	PE9	PE10	PE11	PE12	PE13
$\mu\text{g}/\text{kg}$	11.22	26.49	<LOQ	29.87	18.65	21.71	<LOQ	9.71	9.74	14.78	12.88	21.84	16.13
SD (\pm)	2.24	5.30		2.99	3.73	4.34		1.94	1.95	2.96	2.58	4.37	3.23
	PE14	PE15	PE16	PE17	PE18	PE19	PE20	PE21	PE22	PE23	PE24	PE25	PE26
$\mu\text{g}/\text{kg}$	13.04	<LOD	9.38	12.29	14.83	10.49	6.16	7.45	15.40	1.08	3.30	32.73	42.86
SD (\pm)	2.61		1.88	2.46	2.97	2.10	1.23	1.49	3.08	0.22	0.66	3.27	4.29