Monitoring of acrylamide carcinogen in selected heat-treated foods from Saudi Arabia

Mohammad Rizwan Khan¹ · Zeid Abdullah Allothman¹ · Mu Naushad¹ · Ahmed Khodran Alomary¹ · Sulaiman Mohammed Alfadul²

Received: 4 October 2017 / Revised: 7 January 2018 / Accepted: 14 March 2018 / Published online: 23 March 2018
© The Korean Society of Food Science and Technology and Springer Science+Business Media B.V., part of Springer Nature 2018

Abstract The present study reports the outcomes of assessment on acrylamide levels in selected heat-treated foods of diverse brands and origins from Saudi Arabia. In chips, acrylamide level was detected from 28 to 954 µg/kg, sample 7 (salted) contained higher amount (954 µg/kg) whereas, sample 8 (labneh and mint) comparatively produced lower amount (28 µg/kg). Nuts and dried fruits have generated acrylamide from 2 to 93 µg/kg, salted peanut of Indian origin produced higher amount (93 µg/kg) while apricot (plain) relatively generated lower amount (2 µg/kg). The levels of acrylamide in biscuits, pastry, cacao, chocolate, olive, cheese, corn, oat and wheat flakes, and bread were found from 26 to 234 µg/kg. Biscuits generated high concentration (234 µg/kg) while corn flakes fairly generated lower amount (26 µg/kg). The obtained results have shown a great variation of acrylamide content and reason might be due to foods type, cooking ingredients and, cooking methods, time and temperature.

Keywords Acrylamide · Carcinogen · Foods · Saudi Arabia

Introduction

Since 1950s, the acrylamide has been used in various industries for instance cosmetic, textile, water treatment, oil, paper, dye and plastic [1, 2]. In April 2002, the Swedish National Food Administration and the University of Stockholm have announced that the acrylamide is generated in a huge number of carbohydrate rich foods for instance French fries, potato chips and cereals when cooked at or higher than 120 °C cooking temperature [3]. Later on, the Swedish obtained results were authenticated in a number of other European countries and in the United States of America. At that time, the obtained acrylamide concentrations were significantly superior to the concentrations suggested by the World Health Organization (WHO) [4].

Acrylamide is known to be a thermally processed food toxicant, the major pathway for acrylamide occurrence in foods is the Maillard reaction (a non-enzymatic reaction that provides food its color and aroma during roasting, frying and baking) between amino acid (asparagines) and reducing sugars (glucose and fructose) at high cooking temperature [5, 6]. A number of toxicological investigations have revealed the acrylamide genotoxic carcinogenicity in investigational animals [7, 8]. Based on acrylamide carcinogenicity in experimental animals, the International Agency for Research on Cancer (IARC) and the Environmental Protection Agency (EPA) have considered and categorized as a *probable human carcinogen* [1, 9, 10]. Lately, the European Food Safety Agency (EFSA) has stated that the heat-treated carbohydrate rich foods for instance potato chips, French fries, cookies, crackers, breakfast cereals and toasted bread are the major source of acrylamide which supplied more than 90% ingestion through our foods [11, 12]. The Food and

✉ Mohammad Rizwan Khan
mrkhan@ksu.edu.sa

¹ Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia

² King Abdulaziz City for Science and Technology, P.O. Box 6086, Riyadh 11442, Kingdom of Saudi Arabia

Agriculture Organization (FAO) and WHO have affirmed that the amounts of acrylamide in food products create a most important disquiet and that further study is required to find out the threat of nutritional acrylamide exposure [13]. As compared with other food products, the potato chips and French fries were found to comprise high amounts of acrylamide and in some cases the concentrations reached between 4500 and 12000 µg/kg [14, 15]. The other food products like biscuits, cereal, crackers, nuts and dried fruits contained low amounts of acrylamide and the levels reached up to 3200 µg/kg [16]. So far, numerous information regarding acrylamide occurrence in foods have been accomplished and incorporated in the Food Drug Administration (FDA) [17]. Saudi Arabia is the biggest and an emergent marketplace for high value food products in the Gulf States and relies on foreign dealers to satisfy nearly 80% of its food consumption requirements [18]. Owing to changing way of life and diets in young populations are associated with the consumption of higher quantities of fast foods together with potato chips, French fries, nuts, dried fruits, biscuits, pastry, cacao, chocolate, olive, cheese, corn, oat and wheat flakes, and bread which usually supplies to higher levels of acrylamide intake [19, 20]. Such types of heat-treated foods are frequently present in the Saudi Arabian diets.

Due to low levels of acrylamide in cooked food products, it is greatly challenging for researchers to assess acrylamide in highly matrix foods. Thus, an accurate extraction procedure is needed to efficiently determine the acrylamide levels present in cooked foods. Until now, various extraction techniques have been optimized which involve several clean-up steps, for instance pressurized liquid extraction [21], solid-phase extraction (SPE) [22, 23], and liquid-liquid extraction [24]. In the recent years, a standard method based on SPE has been broadly used [23], and for the elimination of a highly complex matrix, the SPE technique is recognized to be one of the most proficient methods. Relating to the chromatographic separation techniques, primarily liquid chromatography-mass spectrometry (LCMS) technique is presently applied [21–23] for the analysis of acrylamide in foods. This method offers both quantitative and qualitative results, and acrylamide separation can be obtained within 5 min. Most recently, an innovative technique based on ultra-performance liquid chromatography/mass spectrometry (UPLC-MS/MS) has been developed, and has advantages over conventional techniques, i.e., fast analysis (< 1 min) with greater sensitivity, high resolution and lower solvent consumption [25].

In order to make the risk estimation and to assist the consumer selection for the healthy foods in their everyday life, it is highly essential to monitor the presence of acrylamide in Saudi Arabian foods. Therefore, in the present

study, the presence of acrylamide in various Saudi Arabian food products has been studied using SPE and UPLC-MS/MS [23, 25]. The obtained results from the present study will enhance the awareness about acrylamide toxicant in the Saudi populations including worldwide population; in addition it can be employ to estimate the intake of acrylamide toxicant from such kind of food products.

Materials and methods

Materials

Acrylamide (assay $\geq 99.8\%$), acrylamide-2,3,3-D₃ (AA-D₃, isotopic purity 98%), zinc acetate dihydrate (assay $\geq 98\%$) and potassium hexacyanoferrate (assay $\geq 99\%$) were supplied from Sigma-Aldrich (Steinheim, Germany). HPLC-grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). For the purification of water, a Milli-Q water purification system, model advantage A10 from Millipore Corporation (Bedford, USA) was used. Acrylamide stock solutions (10 µg/mL) and AA-D₃ (10 µg/mL) were prepared with Milli-Q water. To obtain a sequence standard solution, the stocks solutions were diluted with Milli-Q water. About the linearity of the method, the calibration curve at different concentrations (8–2000 µg/L) was constructed. All solutions were stored at + 4 °C until analysis. To obtain the Carrez I and II solutions, zinc acetate dihydrate (24 g) and potassium hexacyanoferrate (10.6 g) were separately dissolved in Milli-Q water (100 mL).

For the extraction of acrylamide from food samples, the SPE column Isolute® ENV+ (200 mg, 3 mL) was purchased from Biotage (Uppsala, Sweden) and strata™-X-C polymeric strong cation (200 mg, 6 mL) was supplied from Phenomenex (Torrance, USA). For the sample filtration, polytetrafluoroethylene (PTFE) syringe filters of pore size 0.45 µm were obtained from Macherey–Nagel (Düren, Germany) and nitrocellulose membrane pore size 0.45 µm was obtained from Sigma-Aldrich (Steinheim, Germany).

Microtron® MB800 (Kinematica AG, Littau, Switzerland), ultra-turrax T25 digital (IKA®-WERKE GmbH, Staufen, Germany) and stardust coffee grinder, model CML-1000MKII (Osaka, Japan) were used for the blending and homogenization of food samples. The vacuum manifolds, Visiprep™ and Visidry™ (Supelco, Gland, Switzerland) were applied for SPE and solvent evaporation uses, respectively.

Sample preparation

Heat-treated foods (potato chips, French fries, nuts, dried fruits, biscuits, pastry, cacao, chocolate, olive, bread,

cheese and, corn, oat and wheat flakes) were obtained from local superstores (Riyadh, Saudi Arabia). The foods were mixed and homogenized using various equipments (Microtron[®] MB800, coffee grinder and ultra-turrax T25 digital). The fibrous foods material greater than 250 µm in size were divided, ground and sieved at 250 µm. Finally, the sieved food materials were thoroughly homogenized followed by bottling and labeling. The food samples were refrigerated at − 30 °C until analysis.

Sample extraction

For the acrylamide contents, various heat-treated foods including potato chips, French fries, nuts, dried fruits, biscuits, pastry, cacao, chocolate, olive, bread, cheese and, corn, oat and wheat flakes were studied using previously developed SPE method [22, 23]. In brief, subsamples (2 g) were weighed into 50 mL falcon tube followed by the addition of water (10 mL) and 93 µL of AA-D₃ (internal standard, 10 µg/mL). The sample containing falcon tube was shaken using Stuart tube rotator (Staffordshire, United Kingdom) for 1 h. Afterward, samples were centrifuged at 5000 rpm for 30 min by a centrifugation system, HERMLE model Z32 HK (Wehingen, Germany). For the precipitation of interfering materials in the sample matrix, a clear sample supernatant was taken into a new falcon tube and treated with 500 µL of Carrez I and II solutions, correspondingly. The treated samples were again centrifuged at 4000 rpm for 3 min. After that, an aliquot (3 mL) of the aqueous sample solution was filtered and loaded onto SPE cartridge (StrataTM-X-C polymeric strong cation) which was linked online with a vacuum manifold (VisiprepTM). Subsequently, the SPE StrataTM-X-C column was eluted with 3 mL water and the obtained eluent was loaded onto Isolute[®] ENV+ SPE column and eluted with a mixture of methanol and water at the ratio of 60:40, v/v (1 mL). The final sample extracts were tenderly evaporated to 400 µL by means of vacuum manifold (VisidryTM) under nitrogen stream. Lastly, the samples were filtered through PTFE syringe filters (0.45 µm) and transferred into amber glass vials for UPLC-MS/MS analysis [25].

The quantitation of acrylamide in food samples were performed by the isotope dilution technique (a procedure of quantifying the concentration of chemical substances in highly matrix samples). This technique comprises the adding of known amounts of isotopically-enriched substance to the unknown studied samples. This method supplies further precise outcomes because of an improvement of both extraction efficiency and alters in the machine response. In this work, AA-D₃ as the labeled substance was used to quantify the acrylamide in food samples. Analytes response curve was constructed of area response ratio for

m/z , 55/58 (Table 1) vs the amount of acrylamide injected with a constant amount of AA-D₃ [22, 23].

Instrumentation

In the current investigation, the acrylamide levels in a variety of heat-treated foods were quantitatively and qualitatively analyzed using Waters Acquity[®] UPLC technique equipped with a thermostat auto sampler and column compartment, quaternary pump and vacuum degasser (Milford, USA). The Waters Acquity[®] reversed phase BEH C₁₈ column of dimension 50 mm × 2.1 mm i.d. and particle size, 1.7 µm was applied (Milford, USA) to acquire the separation of both acrylamide and AA-D₃ compounds. The optimal separation of acrylamide and AA-D₃ was accomplished in isocratic elution form by means of mobile phase, 0.1% of formic acid in Milli-Q water (89.90%) and methanol (10%). The mobile phase flow speed and sample injection volume was 0.3 mL/min and 0.005 mL, respectively [25]. So as to achieve highest instrumental sensitivity, at every twenty sample injections, the BEH C₁₈ column was wash with a mixture of water and methanol (50:50, v/v).

The UPLC device was equipped with electrospray ionization source (ESI) and Quattro Premier triple quadrupole mass analyzer (Micromass, Milford, USA). To acquire the utmost instrumental response regarding the analysis of acrylamide and AA-D₃, the mass spectrometric system was operated in positive ionization mode and data were achieved multiple reaction monitoring (MRM) mode. The ESI source operational conditions were as follows: source temperature, 120 °C; desolvation temperature, 350 °C; desolvation gas flow rate, 600 L/h; cone gas flow rate, 60 L/h; capillary voltage, 3.5 kV and cone voltage, 48 V. For the purpose of cone and collision gases, nitrogen gas (purity > 99.99%), produced by a Peak Scientific nitrogen generator, model NM30LA (Inchinnan, United Kingdom) and argon were applied, respectively. To provide the main vacuum to the MS device, an Oerlikon rotary pump, model SOGEVACSV40 BI (Cedex, France) was used. The MS/MS parameters for example collision energy voltages, dwell times and, precursors and daughter ions linked to the selected transitions of acrylamide and AA-D₃ are illustrated in Table 1. For the assessment of acrylamide and AA-D₃, the most abundant daughter ions were selected nonetheless for the confirmation of acrylamide and AA-D₃, the second-most abundant daughter ions were chosen. For the data acquisition, Waters MassLynx V4.1 software (Milford, USA) was used [25].

Table 1 MRM conditions applied with the triple quadrupole mass spectrometric system

Compound	Precursor ion $[M + H]^+$ (m/z)	Quantification		Confirmation	
		Product ion (m/z)	Collision energy (eV)	Product ion (m/z)	Collision energy (eV)
Acrylamide	72	55	48	44	43
Acrylamide-2,3,3-D ₃	75	58	52	47	47

Dwell time (0.025 s) in both cases

Results and discussion

For the identification of acrylamide in heat-treated food products, the quality parameters for instance limit of detection (LOD), limit of quantification (LOQ), linearity and precisions (run-to-run and day-to-day) were studied. The LOD (signal-to-noise ratio, 3:1) and LOQ (signal-to-noise ratio, 10:1) were calculated and acquired 3 and 10 $\mu\text{g/L}$, respectively. Calibration curve based on the ratio of peak areas were built up and it was linear across the studied level ranged between 8 and 2000 $\mu\text{g/L}$ and the correlation coefficients (R^2) was greater than 0.999. To establish the precisions (run-to-run and day-to-day), five replicates of a known amount of standard solution was analyzed on the same day for run-to-run precision and five replicates of same standard solution was injected over three following days for day-to-day precision. The achieved relative standard deviation values were lower than 4%. The achieved values have authenticated that the system can be applied effectively for the precise analysis of acrylamide.

Recovery procedure was established in each food sample using the procedure of standard addition. The known acrylamide standard was spiked to each food sample at a certain amount, which was very close to the obtained acrylamide concentration of the subsequent food samples. The total acrylamide concentrations were established for each food sample, and plotted against the added amounts of acrylamide. Through the linear regression analysis, the y intercept was determined, which demonstrate the incurred amount of acrylamide in the non-spiked food samples. Subtracting the incurred amount of acrylamide from the entire concentration of acrylamide illustrated the recovery of each food sample.

In this study, a total of sixty four food samples of diverse brands and origins were selected for the determination of acrylamide. About the levels of acrylamide in chips and French fries, onion rings and popcorn, a total of thirty four samples were analyzed. The achieved results have been demonstrated in Table 2. The acrylamide was obtained in all of the studied samples and its levels ranged from 28 to 954 $\mu\text{g/kg}$, the utmost levels of acrylamide (954 $\mu\text{g/kg}$) were obtained in sample 7 (salted) while, sample 8 (labneh and mint) relatively formed low amounts

(28 $\mu\text{g/kg}$). To demonstrate the results, the UPLC-MS/MS chromatogram of acrylamide (sample 7, salted) has been shown in Fig. 1. It can be observed that the system provides excellent sensitivity and selectivity during acrylamide identification, while four MRM transitions were attained at the same time. As observed in the previous literatures [11, 12, 16, 22, 26, 27], the highest amount of acrylamide in chips, French fries, popcorn and onion rings was obtained at 4215, 4653, 171 and 202 $\mu\text{g/kg}$, respectively. According to the EFSA, the minimum and maximum acrylamide concentrations for chips and French fries vary from 273 to 4804 $\mu\text{g/kg}$ and 280 to 3380 $\mu\text{g/kg}$, respectively [11]. In addition, the European Union database [27] has also illustrated that the minimum and maximum acrylamide concentrations for chips and French fries vary from 5 to 4215 $\mu\text{g/kg}$ and 5 to 4653 $\mu\text{g/kg}$, respectively. These values are found similar to those achieved in the present study. The concentration variations among analyzed samples might be due to the cooking temperature, time and method, kind of foods (amounts of asparagines and sugars), type of food additives, storage temperature of the raw foods that could influence the acrylamide level [26, 28].

About nuts and dried fruits, nineteen samples have been studied for the acrylamide contents, results are presented in Table 3. The food samples were thermally processed using either roasted or smoked cooking methods. The acrylamide was detected in all of the analyzed samples and its levels ranging from 2 to 93 $\mu\text{g/kg}$, the lowest amount acrylamide was detected in plain apricots (2 $\mu\text{g/kg}$) while the highest amount of acrylamide was detected in slated peanut of Indian origin (93 $\mu\text{g/kg}$). From the obtained results, it can also be observed that some samples processed using smoked cooking condition comparatively generate lower amount of acrylamide than those processed using roasted cooking method. These cause might be due to the smoked food was not directly in contact with heating source which favor the acrylamide formation. Previously, a limited study on the amounts of acrylamide in few food matrices (roasted hazelnut, almond, cashew and peanut) was reported by Olmez et al. [12]; Bermudo et al. [23]; De Paola et al. [29] and the concentration of acrylamide was detected between 10 and 313 $\mu\text{g/kg}$. The values obtained in present study

Table 2 Amounts of acrylamide and recovery rates in potato chips and French fries

Sample	Type	Additives	Country of origin	Before addition, acrylamide ($\mu\text{g/kg}$) $\pm$ SD	Added acrylamide ($\mu\text{g/kg}$)	After addition, acrylamide ($\mu\text{g/kg}$) $\pm$ SD	Recovery rates (%)
Sample 1	Potato	Cheddar cheese	KSA	122 $\pm$ 11	120	233 $\pm$ 20	96
Sample 2	Potato	Salt	Malaysia	652 $\pm$ 24	650	1280 $\pm$ 36	98
Sample 3	Potato	Salt	UAE	236 $\pm$ 12	230	448 $\pm$ 20	96
Sample 4	Potato	Salt	UAE	356 $\pm$ 12	350	680 $\pm$ 22	96
Sample 5	Potato	Cheddar cheese	KSA	96 $\pm$ 5	90	177 $\pm$ 10	95
Sample 6	Dehydrated potato	Salt	KSA	54 $\pm$ 3	50	101 $\pm$ 7	97
Sample 7	Potato	Salt	KSA	954 $\pm$ 35	950	1870 $\pm$ 65	98
Sample 8	Baked Potato	Labneh and Mint	KSA	28 $\pm$ 2	25	51 $\pm$ 5	95
Sample 9	Corn	Salt	KSA	86 $\pm$ 7	80	158 $\pm$ 10	95
Sample 10	Corn	Salt	KSA	140 $\pm$ 10	130	259 $\pm$ 19	96
Sample 11	Corn	Cheese	KSA	69 $\pm$ 6	65	127 $\pm$ 10	95
Sample 12	Onion	Salt	KSA	340 $\pm$ 15	330	645 $\pm$ 28	98
Sample 13	Corn	Cheese	UAE	135 $\pm$ 12	130	252 $\pm$ 22	95
Sample 14	Potato	Salt	Poland	436 $\pm$ 22	400	829 $\pm$ 35	99
Sample 15	Potato	Sour cream and Onion	Belgium	94 $\pm$ 10	90	175 $\pm$ 18	95
Sample 16	Potato	Salt	Belgium	665 $\pm$ 25	650	1305 $\pm$ 46	99
Sample 17	Potato	Cheddar	Germany	190 $\pm$ 12	180	352 $\pm$ 20	95
Sample 18	Potato	Sea salt	Germany	870 $\pm$ 18	860	1697 $\pm$ 33	98
Sample 19	Potato	Salt	KSA	540 $\pm$ 18	530	1028 $\pm$ 32	96
Sample 20	Potato	Salt	KSA	465 $\pm$ 17	460	878 $\pm$ 30	95
Sample 21	Potato	Ketchup	KSA	86 $\pm$ 7	80	158 $\pm$ 10	95
Sample 22	Corn	Salt	KSA	42 $\pm$ 3	40	80 $\pm$ 7	98
Sample 23	Corn	Chili and Lemon	KSA	91 $\pm$ 7	90	172 $\pm$ 11	95
Sample 24	Potato	Cheese	UK	110 $\pm$ 10	105	206 $\pm$ 16	96
Sample 25	Potato	Paprika	Germany	68 $\pm$ 5	60	123 $\pm$ 8	96

Table 2 continued

Sample	Type	Additives	Country of origin	Before addition, acrylamide ($\mu\text{g/kg}$) $\pm$ SD	Added acrylamide ($\mu\text{g/kg}$)	After addition, acrylamide ($\mu\text{g/kg}$) $\pm$ SD	Recovery rates (%)
Sample 26	Potato	Sea salt and Pepper	Germany	210 $\pm$ 11	200	394 $\pm$ 18	96
Sample 27	Potato	Salt	Germany	682 $\pm$ 25	670	1328 $\pm$ 39	98
Sample 28	Potato	Salt	KSA	805 $\pm$ 30	800	1557 $\pm$ 52	97
Sample 29	Potato	Salt and Vinegar	Germany	390 $\pm$ 13	380	755 $\pm$ 22	98
Sample 30	Corn	Honey	KSA	143 $\pm$ 11	140	269 $\pm$ 17	95
Sample 31	Potato	Salt	Bulgaria	62 $\pm$ 3	60	121 $\pm$ 4	99
Sample 32	Potato	Salt	USA	82 $\pm$ 6	80	157 $\pm$ 15	97
Sample 33	Potato	Cheddar and Caramelized onion	USA	40 $\pm$ 3	40	76 $\pm$ 7	95
Sample 34	Potato	Salt	KSA	367 $\pm$ 16	360	713 $\pm$ 24	98

SD, standard deviation ($n = 3$); UAE, United Arab Emirates; KSA, Kingdom of Saudi Arabia; UK, United Kingdom; USA, United States of America

Fig. 1 UPLC-MS/MS chromatograms of acrylamide and acrylamide-2,3,3- D_3 (I. S.) in chips (sample 7, salted)

were found in good agreement and fall in between previously detected acrylamide concentrations [12, 23, 29]. In pine nuts, walnuts and raisins, De Paola et al. [29] has reported that the acrylamide concentration was found

below the limit of detection, whereas, Atwa et al. [30] have detected the acrylamide in pine nuts only and the concentration ranging from 144 to 1845 $\mu\text{g/kg}$. The acrylamide amounts in pine nuts, walnuts and raisins are

Table 3 Amounts of acrylamide and recovery rates in nuts and dried fruits

Sample	Type	Country of origin	Cooking method	Before addition, acrylamide ($\mu\text{g/kg}$) $\pm$ SD	Added acrylamide ($\mu\text{g/kg}$)	After addition, acrylamide ($\mu\text{g/kg}$) $\pm$ SD	Recovery rates (%)
Peanut	Plain	India	Roasted	73 $\pm$ 4	70	137 $\pm$ 7	96
Peanut	Salted	India	Roasted	93 $\pm$ 6	90	181 $\pm$ 10	99
Peanut	Lemon	India	Roasted	72 $\pm$ 3	70	135 $\pm$ 5	95
Peanut	Salted	China	Roasted	13 $\pm$ 5	10	22 $\pm$ 7	96
Peanut	Plain	China	Smoked	18 $\pm$ 4	15	32 $\pm$ 6	97
Hazelnuts	Plain	Turkey	Smoked	68 $\pm$ 4	65	132 $\pm$ 7	99
Pine Nuts	Plain	Pakistan	Roasted	82 $\pm$ 5	80	159 $\pm$ 7	98
Walnuts	Plain	USA	Roasted	74 $\pm$ 4	70	138 $\pm$ 7	96
Pecans	Salted	USA	Roasted	79 $\pm$ 4	75	151 $\pm$ 6	98
Almond	Salted	USA	Roasted	23 $\pm$ 2	20	41 $\pm$ 3	95
Almond	Lemon	USA	Roasted	36 $\pm$ 2	35	68 $\pm$ 3	96
Almond	Salted	USA	Smoked	12 $\pm$ 1	10	21 $\pm$ 2	95
Pistachio	Plain	USA	Roasted	24 $\pm$ 2	20	43 $\pm$ 4	98
Pistachio	Salted	USA	Roasted	74 $\pm$ 5	70	141 $\pm$ 10	98
Pistachio	Lemon	USA	Roasted	72 $\pm$ 4	70	136 $\pm$ 7	96
Cashew	Plain	USA	Roasted	35 $\pm$ 2	35	67 $\pm$ 3	96
Cashew	Salted	USA	Roasted	81 $\pm$ 6	80	155 $\pm$ 10	96
Raisins	Plain	Iran	–	5 $\pm$ 0.2	5	9.5 $\pm$ 0.3	95
Apricots	Plain	Turkey	–	2 $\pm$ 0.1	2	3.8 $\pm$ 0.2	95

USA, United States of America; –, not described; SD, standard deviation (n = 3)

completely differing with those obtained in earlier studies [29, 30], however, they are under permissible limits as recommended by the WHO. We have detected the amount of acrylamide in pistachio at 72 $\mu\text{g/kg}$, however in some study the value goes up to 462 $\mu\text{g/kg}$ [31]. About pecans and apricot, this is the first study relating to the analysis of acrylamide and the detected concentrations reached up to 79 $\mu\text{g/kg}$. The amount difference among analyzed samples might be due to the cooking temperature, time and method, kind of foods (amounts of asparagines and sugars), type of food additives, and storage temperature of the raw foods that could influence the acrylamide level [26].

Relating to the acrylamide contents in biscuits, pastry, cacao, chocolate, olive, bread, cheese and, corn, oat and wheat flakes, a total of eleven food samples have been investigated, results are shown in Table 4. The acrylamide has been detected in all of the analyzed samples and the concentrations were found in the range of 26–234 $\mu\text{g/kg}$. In biscuits, the levels of acrylamide were found at 234 $\mu\text{g/kg}$, these values were agreeable with acrylamide amounts found in earlier studies [12, 23, 32]. Relating to the pastry, the concentrations of acrylamide was detected at 72 $\mu\text{g/kg}$ and found in good agreement with those values obtained in the earlier study with a minimum amount of 40 $\mu\text{g/kg}$ and maximum amount of 248 $\mu\text{g/kg}$ [21]. In cacao and

chocolate, the acrylamide levels were achieved at 92 and 84 $\mu\text{g/kg}$, respectively. In both cases, the similar cooking methods were applied and found no great variations in their concentrations. In cacao, Al-Dmoor [33] has detected acrylamide levels at 1100 $\mu\text{g/kg}$ which is very far from the amounts we obtained in the present study. These high amounts can be recognized to the extent of very bad cooking conditions (roasting) which proved to be valuable cause in acrylamide formation. In chocolate, Olmez et al. [12] have detected the amounts of acrylamide in the range of 37–100 $\mu\text{g/kg}$ and found in concurrence with those values obtained in the present study. The olives are the Mediterranean product that can be eaten either as olive oil or as a whole after some treatment. Previously, various analyses were carried out by the FDA [17] and illustrated that the black olives offered comparatively higher amount of acrylamide. In the present study, two types of olive samples (green and black) have been analyzed and their concentrations reached between 32 and 97 $\mu\text{g/kg}$, correspondingly. Black olives contained the highest amount, although these amounts were usually lower than those presented in earlier investigations [17, 22, 32]. In cheese, the levels of acrylamide was obtained at 47 $\mu\text{g/kg}$ which is lower than the amounts presented in the previous study [17], where the minimum and maximum values ranging

Table 4 Amounts of acrylamide and recovery rates in miscellaneous food products

Sample	Country of origin	Cooking method	Before addition, acrylamide ($\mu\text{g/kg}$) $\pm$ SD	Added acrylamide ($\mu\text{g/kg}$)	After addition, acrylamide ($\mu\text{g/kg}$) $\pm$ SD	Recovery rates (%)
Biscuits	UK	Baked	234 $\pm$ 12	230	455	98
Pastry (light Chocolate)	KSA	Baked	72 $\pm$ 3	70	135	95
Cacao	Malaysia	Roasted	92 $\pm$ 4	90	180	99
Chocolate	Singapore	Roasted	84 $\pm$ 4	80	161	98
Olive (green)	Spain	–	32 $\pm$ 2	30	60	97
Olive (black)	Spain	–	97 $\pm$ 4	95	185	96
Cheese	Egypt	–	47 $\pm$ 2	45	88	96
Corn flakes	Poland	–	26 $\pm$ 1	25	50	98
Oat flakes	France	–	145 $\pm$ 7	140	279	98
Wheat flakes	Spain	–	41 $\pm$ 2	40	77	95
Bread	KSA	Toasted	194 $\pm$ 10	190	369	96

UK, United Kingdom; KSA, Kingdom of Saudi Arabia; –, not described; SD, standard deviation ($n = 3$)

from not detected to 196 $\mu\text{g/kg}$. In breakfast cereals (corn, oat and wheat flakes) the acrylamide was determined at the concentrations of 26, 145 and 41 $\mu\text{g/kg}$, respectively. Studies have been previously performed by Lineback et al. [32] and Olmez et al. [12], and data presented in the FDA [17] demonstrates that these food products contained relatively lower concentrations of acrylamide. Relating to the concentrations of acrylamide in toasted bread, in this study we achieved at 194 $\mu\text{g/kg}$. This amount is similar to those obtained in previous studies, Olmez et al. [12] has obtained the minimum and maximum levels of acrylamide in the range of 41–474 $\mu\text{g/kg}$, whereas Lineback et al. [32] have presented the lower and higher values acrylamide in the range of 25–1430 $\mu\text{g/kg}$. The concentrations variation among studied food samples might be due to the cooking temperature, time and method, and type of foods (amounts of asparagines and sugars), type of food additives and storage temperature of the raw foods that could influence the formation of acrylamide [26]. In all of the analyzed food samples, the excellent recovery rates were achieved between 95 and 99%.

In summary, the amounts of acrylamide in sixty four food samples including potato chips, French fries, nuts, dried fruits, biscuits, pastry, cacao, chocolate, olive, bread, cheese and, corn, oat and wheat flakes of different brands and origins were investigated. The acrylamide amounts were obtained between 2 and 954 $\mu\text{g/kg}$. All studied food samples have demonstrated an important source of acrylamide. The chips (sample 7, salted) was found the most contaminated food and the concentrations reached up to 954 $\mu\text{g/kg}$, whereas chips (sample 8, labneh and mint,) has been found the low contaminated ones and the amounts

reached up to 28 $\mu\text{g/kg}$. In French fries the acrylamide levels reached up to 367 $\mu\text{g/kg}$. In nuts, dried fruits and miscellaneous food products (biscuits, pastry, cacao, chocolate, olive, cheese, corn, oat and wheat flakes and bread samples), the amounts of acrylamide were found between 2 and 234 $\mu\text{g/kg}$. Comparatively, the nuts contained higher acrylamide amounts (12–93 $\mu\text{g/kg}$) than the dried fruits (2–5 $\mu\text{g/kg}$). Among miscellaneous food products, the biscuits contained the higher amounts of acrylamide and the concentrations reached up to 234 $\mu\text{g/kg}$, while wheat flake contained the lower amounts of acrylamide (41 $\mu\text{g/kg}$). The variation of acrylamide in such type of carbohydrate-rich foods may caused due to the thermal treatment, the acrylamide formation mechanism revealed that the acrylamide generated at higher level, if foods are thermally processed for longer times at higher temperature. The outcomes disclosed that the acrylamide amounts of thermally treated food products demonstrate a big difference between diverse food group and brands. The obtained data can also be used in epidemiological investigation to estimate the acrylamide exposure from nutritional survey.

Acknowledgements The authors are very thankful to the King Abdulaziz City for Science and Technology (KACST), Kingdom of Saudi Arabia for the financial support of this project (Project Number 193–35).

References

1. EPA. Environmental Protection Agency 749-F-94-005a: Chemical Summary for Acrylamide (1994).

2. Shipp A, Lawrence G, Gentry R, McDonald T, Bartow H, Bounds J, Macdonald N, Clewell H, Allen B, Van Landingham C. Acrylamide: Review of toxicity data and dose-response analyses for cancer and noncancer effects. *Crit. Rev. Toxicol.* 36: 481–608 (2006).
3. SNFA. Analysis of Acrylamide in Food, Swedish National Food Administration, (2002). <http://www.slv.se/acrylamide>.
4. WHO. Acrylamide in Drinking-Water. Background Document for Development of World Health Organization Guidelines for Drinking-Water Quality, Geneva, (2011). http://www.who.int/water_sanitation_health/dwq/chemicals/acrylamide.pdf (Retrieved November 20, 2016).
5. Donald SM, Bronislaw LW, Andrew TD. Food chemistry: Acrylamide is formed in the Maillard reaction. *Nature* 419: 448–449 (2002).
6. Richard HS, Imre B, Natalia V, Fabien R, Jörg H, Philippe AG, Marie-Claude R, Sonja R. Food chemistry: Acrylamide from Maillard reaction products. *Nature* 419: 449–450 (2002).
7. Klaunig JE. Acrylamide carcinogenicity. *J. Agric. Food Chem.* 56: 5984–5989 (2008).
8. Beland FA, Mellick PW, Olson GR, Mendoza MC, Marques MM, Doerge DR. Carcinogenicity of acrylamide in B6C3F(1) mice and F344/N rats from a 2-year drinking water exposure. *Food Chem. Toxicol.* 51: 149–159 (2013).
9. IARC. Acrylamide, in Some Industrial Chemicals. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 60. Lyon, France: International Agency for Research on Cancer, pp 389–433, (1994).
10. IARC. Acrylamide, in Some Chemicals Used in Plastics and Elastomers. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 39. Lyon, France: International Agency for Research on Cancer, pp 41–66, (1986).
11. EFSA. European Food Safety Authority, Draft scientific opinion on acrylamide in food (2014). <http://www.efsa.europa.eu/en/consultations/call/140701.pdf> (Retrieved November 20, 2016).
12. Olmez H, Tuncay F, Ozcan N, Demirel S. A survey of acrylamide levels in foods from the Turkish market. *J. Food Compos. Anal.* 21: 564–568 (2008).
13. FAO/WHO. Summary report of the sixty-fourth meeting of the Joint Food and Agriculture Organization & World Health Organization Expert Committee on Food Additives (2002). <http://www.who.int/entity/ipcs/food/jecfa/summaries/summaryreport64final.pdf> (Retrieved November 20, 2016).
14. EFSA. Results on acrylamide levels in food from monitoring years 2007–2009 and exposure assessment, Scientific Report of European Food Safety Authority Journal, 9: 2133. (2011).
15. Friedman M. Chemistry, biochemistry, and safety of acrylamide. A review. *J. Agric. Food Chem.* 51: 4504–4526 (2003).
16. Hoenicke K, Gattermann R, Harder W, Hartig L. Analysis of acrylamide in different foodstuffs using liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry. *Anal. Chim. Acta*, 520: 207–215 (2004).
17. FDA. Survey Data on Acrylamide in Food: Individual Food Products. Food Drug Administration (2006). <http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm053549.htm> (Retrieved November 1, 2012).
18. FAS. Foreign Agricultural Service (2015). http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Exporter%20Guide/Riyadh_Saudi%20Arabia10-1-2015.pdf (Retrieved November 20, 2016).
19. Wyka J, Tajner-Czopek A, Broniecka A, Piotrowska E, Bronkowska M, Biernat J. Estimation of dietary exposure to acrylamide of Polish teenagers from an urban environment. *Food Chem. Toxicol.* 75: 151–155 (2015).
20. Mesias M, Morales FJ. Acrylamide in commercial potato crisps from Spanish market: Trends from 2004 to 2014 and assessment of the dietary exposure. *Food Chem. Toxicol.* 81: 104–110 (2015).
21. Yusà V, Quintás G, Pardo O, Martí P, Pastor A. Determination of acrylamide in foods by pressurized fluid extraction and liquid chromatography-tandem mass spectrometry used for a survey of Spanish cereal-based foods. *Food Addit. Contam.* 3: 237–244 (2006).
22. Bermudo E, Moyano E, Puignou L, Galceran MT. Determination of acrylamide in foodstuffs by liquid chromatography ion-trap tandem mass-spectrometry using an improved clean-up procedure. *Anal. Chim. Acta* 559: 207–214 (2006).
23. Bermudo E, Moyano E, Puignou L, Galceran MT. Liquid chromatography coupled to tandem mass spectrometry for the analysis of acrylamide in typical Spanish products. *Talanta* 76: 389–394 (2008).
24. Biedermann M, Biedermann-Brem S, Noti A, Grob K, Egli P, Mandli H. Two GC-MS methods for the analysis of acrylamide in food. *Mitt. Lebensm. Hyg.* 93: 638–652 (2002).
25. Zhang Y, Jiao J, Cai Z, Zhang Y, Ren Y. An improved method validation for rapid determination of acrylamide in foods by ultra-performance liquid chromatography combined with tandem mass spectrometry. *J. Chromatogr. A* 1142: 194–198 (2007).
26. Murkovic M. Acrylamide in Austrian foods. *J. Biochem. Biophys. Methods* 61: 161–167 (2004).
27. JRC. Acrylamide Level Monitoring Database Overview of 5 years of data collection in Europe, Joint Research Centre, Institute for Reference Materials and Measurements (2008).
28. Ghiasvand AR, Hajipour S. Direct determination of acrylamide in potato chips by using headspace solid-phase microextraction coupled with gas chromatography-flame ionization detection. *Talanta* 146: 417–422 (2016).
29. De Paola, EL, Montevicchi G, Masino F, Garbini D, Barbanera M, Antonelli A. Determination of acrylamide in dried fruits and edible seeds using QuEChERS extraction and LC separation with MS detection. *Food Chem.* 217: 191–195 (2017).
30. Atwa MA, Emara MF, Hamza AS, Elmeleigy KM. Acrylamide levels in heat-treated Egyptian foods. *J. Food Dairy Sci.* 1: 69–84 (2010).
31. EC. European Commission recommendation of 10.1.2011 on investigations into the levels of acrylamide in food (2011). http://ec.europa.eu/food/safety/docs/cs_contaminants_catalogue_acrylamide_recommendation_10012011_food_en.pdf (Last accessed 29 September 2016).
32. Lineback DR, Coughlin JR, Stadler RH. Acrylamide in foods: a review of the science and future considerations. *Annu. Rev. Food Sci. Technol.* 3: 15–35 (2012).
33. Al-Dmoor HM. Determination of acrylamide levels in selected traditional foodstuffs and drinks in Jordan. *J. Food Agric. Environ.* 3: 77–80 (2005).