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Determination of acrylamide in potato chips by a reversed-phase LC–MS method based on a stable isotope dilution assay

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Abstract

Potato-based products represent an important part of the daily intake of food-derived acrylamide, mainly on adolescent population from western countries. A reversed-phase liquid chromatography-mass spectrometry based on a stable isotope dilution assay was used for acrylamide analysis. Aqueous sample extraction, cleaning with Carrez solution and solid phase extraction with methanol was applied. The ratio potato/NaCl solution is critical during extraction where the optimum ratio is 0.125 g/ml NaCl 2 M solution. The use of virgin olive oil, as retaining matrix, during methanol desiccation was critical to achieve high recoveries. The method performance was validated for limit of detection (23.2 μg/kg) and quantitation (91.8 μg/kg), linearity ($r > 0.999$, 25–1000 μg/kg), recovery (98.8%). The method was applied on commercial potato chips; the intra-day repeatability was set at 3.9% and values were corrected with a labeled internal standard ($^{13}C_3$-acrylamide). No significant differences on the acrylamide content were observed between industrial-scale and local-scale processed potato chips.

Keywords: Acrylamide; Potato chips; Stable isotope dilution analysis; LC–MS

1. Introduction

Acrylamide is a useful industrial chemical that was labeled as a probable human carcinogen by the International Agency for Research on Cancer (IARC). In this way, the contamination of drinking water or plants grown hydroponically has been the driving force in the past to develop methods for acrylamide monomer analysis (Castle, Campos, & Gilbert, 1991; Hashimoto, 1976). Things changed recently, when in April 2002, researchers from the University of Stockholm and the Swedish National Food Administration (NFA) reported the presence of acrylamide in a wide range of fried and oven-cooked foods, most notably in potato chips and French fries, at levels of 224–3700 μg/kg (Swedish National Food Administration, 2002; Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). Besides, Mottram and Wedzicha (2002) showed how acrylamide could be formed from food components during heat treatment as a result of the Maillard reaction, likely asparagine and glucose. Asparagine, a major amino acid in potatoes and cereals, is a crucial participant in the formation of acrylamide by Maillard reaction at temperatures above 100 °C (Friedman, 2003). Since potato products are especially rich in asparagine and reducing sugars, it is now thought that this Maillard reaction is most likely responsible for the majority of the acrylamide found in potato chips and French fries.

The potential health risk of food acrylamide has been considered by number of government agencies and national authorities (Food Standards Agency, 2002; Scientific Committee on Food, 2002). Subsequently, all available data on acrylamide have been reviewed at international level, e.g., FAO, WHO, JIFSAN

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Workshop- identifying and listing a number of research gaps and priorities (FAO/WHO consultation, 2002; JIFSAN, 2002). Among these, the development and validation of sensitive and reliable analytical methods for the quantification of acrylamide in different food matrices was considered as essential (Pittet, Périsset, & Oberson, 2004).

Because of its high water solubility and high reactivity (Mottram & Wedzicha, 2002) and also, because of the lack of a chromopher group, acrylamide is not easy to detect (Jezussek & Schieberle, 2003). At present, several analytical methods are available for determining acrylamide in foods and the majority are classical methods based on high performance liquid chromatography (LC) or gas chromatography (GC) techniques (Andrzejewski, Roach, Gay, & Musser, 2004; Barber, Hunt, LoPachin, & Ehrlich, 2001; Bologna, Andrews, Barvenik, Lentz, & Sojka, 1999; Castle, 1993; Jezussek & Schieberle, 2003; Kawata et al., 2001; Pittet et al., 2004; Tareke, Rydberg, Karlsson, Eriksson, & Törnvqist, 2000; Tekel, Farkas, & Kovác, 1989; US EPA, 1996). To increase the selectivity and also the sensitivity in GC analysis, bromination of the double bond in combination with electron capture detection was previously applied (Hashimoto, 1976); later, this method was improved by using either methacrylamide or *N*,*N*-dimethylacrylamide as internal standards and mass-spectrometry (MS) as detection method (Ahn et al., 2002; Jung, Choi, & Ju, 2003; Tareke et al., 2000; Tareke et al., 2002) However, several groups also described methods to quantify acrylamide by direct GC–MS measurements without bromination where the loss of acrylamide at the injection port should be evaluated (Biedermann, Biedermann-Brem, Noti, & Grob, 2002; Clarke, Kelly, & Wilson, 2002).

In a recent assessment of the performance of 37 laboratories in determining acrylamide in crisp bread, Clarke et al. (2002) reported that the majority of laboratories use either GC–MS or LC–MS with no obvious method-dependent differences in results obtained between the two approaches. However, the main advantage on LC–MS based methods is that acrylamide can be analyzed without prior derivatization, which considerably simplifies and expedites the analysis (Joint European Commission & Swedish National Administration, 2003). Many laboratories has developed its "own" LC–MS or LC–MS/MS technique (Andrzejewski et al., 2004; Ahn et al., 2002; Becalski, Lau, Lewis, & Seaman, 2003; Jezussek & Schieberle, 2003; Roach, Andrzejewski, Gay, Nortrup, & Musser, 2003; Tareke et al., 2002) but one of the limitations of these techniques is the difficulty of applying an universal clean-up approach valid for many different food matrices that avoid interferences from co-extractives (Presentation at HPLC, 2003).

The purpose of the following investigation was, therefore, to determine the acrylamide content of commercial potato chips as a major source of acrylamide on the diet. In this way, a robust and sensitive LC–MS method was used taking into account different approaches described previously in the literature. Effectiveness of the procedure was evaluated and applied on the study of the content of acrylamide on potato chips.

2. Materials and methods

2.1. Samples

Experiments were conducted with a series of commercial potato chips (39 brands from 34 producers) randomly purchased on different supermarkets ($n = 27$) and fried-potato shops ($n = 12$). Samples (400–800 g) were thinly sliced to assure a homogeneous distribution of hot-spots. A portion (200 g) was distributed in four containers and stored under vacuum and light protected at 4 °C until analysis.

2.2. Chemicals and materials

[$^{13}C_3$]-acrylamide (isotopic purity 99%) was from Cambridge Isotope Labs (Andover, MA, USA). Acrylamide (99%), potassium ferrocianide (Carrez I), zinc acetate (Carrez II)-both analytical-reagent grade- and sodium chloride were from Sigma–Aldrich (St. Louis, MO, USA). Acetic acid (ultrapure grade) was from Merck (Darmstadt, Germany). Methanol and acetonitrile (HPLC grade) were from Scharlau (Barcelona, Spain).

The solid-phase extraction (SPE) cartridges Isolute Multimode (500 mg, 3 ml) were from IST (Hewgoed, Mid-Glamorgan, UK), reversed-phase Oasis HLB (200 mg, 6 ml) and mixed mode cation exchange cartridge Oasis MCX (60 mg, 3 ml) were from Waters (Milford, MA, USA). Syringe filter units (0.45 μm, nylon) were purchased from Tecknokroma (Madrid, Spain).

2.3. Standard and reagents

Stock solutions of acrylamide (0.01 mg/ml) and [$^{13}C_3$]-acrylamide (5 μg/ml) were prepared by dissolving the compounds in Milli-Q water and methanol, respectively. These solutions were then appropriately diluted with Milli-Q water (Millipore Corp., Madrid, Spain) to prepare working standards at 1.0 μg/ml. All stock solutions and working standards were stored light-protected in a refrigerator at 4 °C for maximum 3 months. New working standards were compared with previous one as control of quality. Daily the instrument performance and relative response of labeled (m/z 75.1) and unlabeled (m/z 72.1) acrylamide was verified. Carrez I was prepared by dissolving 15 g of potassium ferrocianide in 100 ml of water and Carrez II by dissolving 30 g of zinc acetate in 100 ml of water.

2.4. Sample extraction

A portion of the sample (1.0 g) was weighed with a precision of 0.1 mg and suspended with 8 ml of sodium chloride 2 M in polypropylene 15 ml centrifugal tubes. Then, the sample was spiked with 200 μl of a 5000 μg/ml [$^{13}C_3$]-acrylamide solution as internal standard and homogenized using a tube shaker. Acrylamide extraction was performed by incubation in a water bath at 60 ± 0.1 °C for 30 min, and 10 s shaking every 10 min. In order to clarify the solution, 1 ml of each Carrez I and Carrez II were added and finally the mixture (10 ml) was centrifuged (9000*g*/10 min/4 °C). In this way, three layers were obtained: a thin fat layer at the top, the aqueous layer in the middle and the lower precipitate layer.

An aliquot (3 ml) of clarified aqueous layer was promptly removed by pipette for SPE clean-up. The pipette was inserted through the top oil layer avoiding the bottom solids layer with the pipette tip. If the 3 ml aliquot was stored at refrigeration previous SPE clean-up, no additional precipitate was observed.

2.5. Sample clean up

In order to clean-up the aqueous extracts, two different SPE cartridges were assayed. Isolute Multimode was preconditioned with 1 ml of methanol and 2×2 ml of water. An aliquot of the clear supernatant (0.5 ml) were passed through the cartridge. Sample (1 ml) was loaded onto the same column and collected by pressure-induced flow.

In other way, Oasis HLB and MCX cartridges were preconditioned with 2 ml of methanol and Oasis HLB cartridge with another 2 ml of Milli-Q water. Then, 1.5 ml of clear supernatant were loaded onto the HLB cartridge and it was washed with 0.75 ml of Milli-Q water. Finally, MCX cartridge was coupled to the HLB one and acrylamide was eluted with 3 ml of methanol, which were eluted through the MCX cartridge.

The Isolute Multimode or Oasis extracts were collected in rotary evaporator heart-flasks and added with 0.4 ml of commercial virgin Spanish olive oil as retaining matrix (other substances could also be used). Olive oil was evaluated for free acrylamide content. Then, the samples were evaporated to dryness on a rotary evaporator (60 °C) or on a heated-block (40 °C) under a stream of nitrogen. Finally, acrylamide was dissolved in 1 ml of mobile phase and olive oil removed with hexane (3×3 ml). The solution was filtered through a 0.45 μm filter into an amberlite LC–MS vial.

2.6. LC–MS analysis

Sample extracts and calibration standards (20 μl) were analyzed on an Agilent 1100 liquid chromatograph coupled to an Agilent Quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Analytical separation was achieved with an Extrasyl ODS1 (20×0.3 cm, 5 μm; Tecknokroma, Madrid, Spain) at 32 °C. Isocratic elution was achieved with a mobile phase of acetic acid–methanol–Milli-Q water (0.1:1.0:98.9) at a flow rate of 0.4 ml min^{-1}.

Electrospray ionization in the positive ionization mode was used. The MS detector operated in selected ion monitoring (SIM) mode at *m*/*z* ratios of 72.1 and 75.1 for acrylamide and labeled [$^{13}C_3$]-acrylamide, respectively. Under these HPLC conditions, acrylamide eluted at 6.8 min. The needle and cone voltages were set at 3.0 kV and 100 V, respectively. Nitrogen was used as nebulizer gas (12.0 l/h) and the source temperature was set at 300 °C.

2.7. Quantitation

Acrylamide was quantified using a linear calibration function that was established with standard solutions of acrylamide and [$^{13}C_3$]-acrylamide dissolved in Milli-Q water at the concentration levels 25, 50, 100, 200, 300, 400, 500, 650, 800 and 1000 μg/l. These concentration values were within the range as encountered in the sample extracts. A calibration graph was constructed by plotting peak area ratios against the corresponding ratios of analyte amounts. Thus, the acrylamide contents in sample extracts were calculated from the calibration slope and intercept value, taking into account the recovery calculated by means of [$^{13}C_3$]-acrylamide slope. In order to perform a good quantification, the signal-to-noise ratio of the LC–MS peak had to be grater than 3:1.

2.8. Statistical

Statgraphics plus v.2.0 (Statistical Graphics Corp., Rockville, MD, USA) was used for statistical analysis of data. All analyses were performed by duplicate from two independent extractions at least.

3. Results and discussion

This work describes a quantitative analytical method for the determination of acrylamide in potato chips in order to estimate the incidence of acrylamide in the consumption of potato-based products (potato crisp and French fries) in the Spanish population. The proposed method is comparable to other different methods described in the literature (Becalski et al., 2003; Biedermann et al., 2002; Roach et al., 2003) but improvements have been made particularly regarding sample extraction and clean-up, i.e., Carrez clarification, addition of olive oil after SPE purification, etc. The most efficient approached described previously by different authors

are evaluated and combined for acrylamide determination in potato-based foodstuffs. Moreover, isotopically-labeled [$^{13}C_3$]-acrylamide is added to the test portion before extraction so as to keep control on the recoveries achieved and to keep track of possible losses occurring during the whole sample pre-treatment (extraction, clean-up and concentration).

3.1. Sample extraction

Extraction of free acrylamide monomer from the food is a critical step since acrylamide may be firmly enclosed and not homogeneously distributed, e.g., in dark solids of potato chips (Biedermann et al., 2002); concentrations are likely to be particularly high in this particles. In this way, method development has to overcome these problems that spiking experiments are not suitable to check extraction of enclosed material.

Different approaches have been described for sample extraction, such as orbital shaker, ultrasonic bath, accelerated solvent extraction, diastase, or freezing. To check for effectiveness of the extraction, the same sample was incubated at different extraction times (Table 1). It was observed that 30 min gave higher acrylamide extraction and that prolonged time did not exert additional advantages. In addition, to know if some acrylamide was retained in the residue the samples were extracted a second time by swollen again the residues from the fist extraction. Extraction was considered complete at 30 min because of the second extract contained less than 10% of the acrylamide concentration of the first extract.

Because of different authors (Castle, 1993; Rosen & Hellenäs, 2002) found acrylamide losses related with the extraction temperature, an experiment in which a potato chip extract added with standard acrylamide was heated at 60 or 80 °C from 20 to 60 min was carried out. No significant acrylamide loss was detected and the extraction of acrylamide was not improved (data not shown). In addition, the effect of sonication over acrylamide extraction was also evaluated. Times of 1, 2 or 5 min did not induce significant acrylamide losses in both acrylamide standard solution and potato sample extract.

Some authors claimed by the use of sodium chloride solution during the extraction of acrylamide (Presentation at HPLC, 2003). In order to evaluate the effectivity of sodium chloride on the extraction of acrylamide in potato-based products, different amounts of sample were extracted with the same volume of sodium chloride (Fig. 1). It could be seen that higher amounts of acrylamide were extracted when higher quantities of potato chips were added until a limit was reached (with 1.00 g of sample). Thus, a linear correlation between acrylamide content and added potato chip was observed from 0.10 to 1.00 g of sample. The amount of sample added was adjusted such that, on the one hand, blending resulted in a paste enabling easy mixing. When mixing resulted in an excessively stiff paste (from 1.25 to 1.50 g of potato chip), lower acrylamide recuperation was observed, 81.4% and 68.5% for 1.25 and 1.50 g, respectively. On the other hand, the sample should not turn as liquid that solids sediment. Then, 1.00 g of sample was selected as a reasonable amount of potato chip to analyze per 8 ml of NaCl solution in the extraction step.

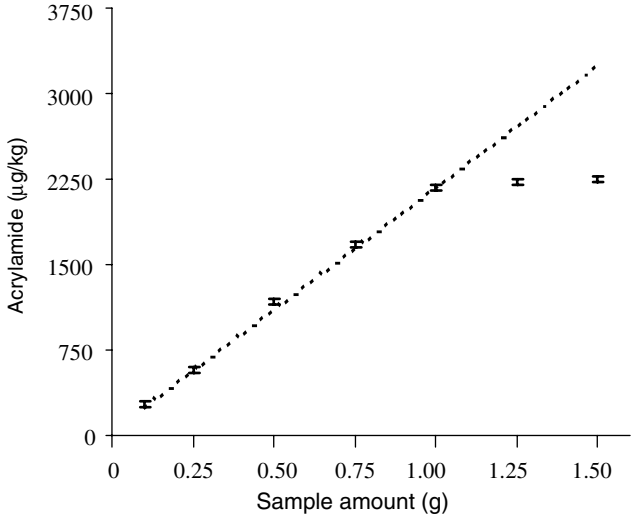

Fig. 1. Influence of sample amount over acrylamide extraction with 8 ml of sodium chloride (2 M). Expected acrylamide content as dotted line.

After sample extraction, in order to obtain a clear supernatant, it was assayed an additional freeze–unfreeze cycle (Riediker & Stadler, 2003). After centrifugation at high speed and low temperature (4 °C) it was observed a cloudy supernatant. In other experiment

Table 1
Effect of the sample extraction time in a water bath at 60 °C on acrylamide analysis[a]

Extraction time (min)	Acrylamide (µg/kg) 1st extraction	Recovery (%)	Acrylamide (µg/kg) 2nd extraction	Recovery (%)	Total recovery (%)
10	506 ± 48	45.0	602 ± 32	53.5	98.5
20	879 ± 31	78.1	254 ± 28	22.6	100.7
30	1129 ± 34	100.3	n.q.[b]	–	100.3
45	1138 ± 28	101.2	n.q.	–	101.2
60	1117 ± 41	99.3	n.q.	–	99.3

[a] Average values from two independent analysis.
[b] Not quantifiable.

Carrez was added to achieve phase separation, to precipitate proteins and other high-molecular co-extractives such as starch. Because of a clear supernatant was obtained, Carrez was chosen as clarification reagent and the freeze–unfreeze cycle was avoided.

3.2. Sample clean up

Preliminary trials with the proposed method by Zyzak et al. (2003) showed the presence of some interfering co-extractives on the LC–MS chromatograms. Therefore, limit of quantification was not acceptable (100 ppb) due to ionic suppression effects. In order to partially overcome this problem, it was found necessary to purify acrylamide extracts by solid-phase extraction (SPE). Two different types of cartridges were assayed, Isolute Multimode and the mixed Oasis HLB + MCX procedure. The characteristic features of the Multimode sorbent are hydrophobic interaction (presence of C_{18} functional groups), strong cationic (SCX) as well as anionic (SAX) exchange. On the other hand, Oasis HLB is a C_{18} cartridge and Oasis MCX a SCX one. In this way, it was minimized the interfering co-extractives load with both types of SPE cartridges, showing "cleaner" chromatograms and higher signal responses due to less ion suppression effects (data not shown).

The removal of both water and methanol solvent coming from Isolute and Oasis cartridges, respectively, was assayed by heating at 40 °C under a stream of nitrogen or by rotary evaporation at 60 °C. Evaporation is a critical step because of acrylamide losses. As shown by Biedermann et al. (2002) acrylamide is largely lost if solvents are completely evaporated without a residue to retain it. We agree with this statement since different evaporation steps were performed in absence of oil founding important losses of acrylamide meaning a dramatic decrease of recoveries. Thus, olive oil was studied as retaining residue. Table 2 shows the results obtained for different combinations of solvent removal with or without olive oil under acrylamide loss of a standard solution. The highest acrylamide losses were obtained when oil was no present. Rotary evaporation showed lower acrylamide losses than nitrogen-heat drying (Table 2) and, in addition, acrylamide was lost in lower amounts when methanol was used as solvent. Solvent removal is a critical step and must be performed under rotary evaporation and oil addition. On the one hand, vastly excessive time may still result in losses of acrylamide, as well as incomplete methanol removal may disturb acrylamide quantification. The whole process was timed (about 4 min) and the last step kept under technical surveillance.

Table 2
Comparison of different solvent removal procedures on the acrylamide recovery in potato chips extracts[a]

Drying method	Solvent	Oil	Recovery (%)
Rotary evaporation	Methanol[b]	–	36.7 ± 4.8
		Yes	96.6 ± 1.1
	Water[c]	–	2.6 ± 5.2
		Yes	53.6 ± 3.1
N_2/Heat	Methanol[b]	–	8.1 ± 4.8
		Yes	42.4 ± 2.9
	Water[c]	–	30.3 ± 1.7
		Yes	59.9 ± 4.3

[a] Average values from two independent analysis.
[b] Extract from Oasis clean-up procedure.
[c] Extract from Isolute clean-up procedure.

Finally, although Isolute Multimode and Oasis showed similar clean-up capacity, it were selected the Oasis cartridges because of the lowest lost of acrylamide during solvent removal and lowest rotary evaporation times. The removed oil by hexane extraction was analyzed for acrylamide content. Thus, 2 ml of water were added to the hexane–oil mixture, shook for 1 min and water extract analyzed by LC–MS. It could be observed no acrylamide presence.

3.3. LC–MS

Different columns were assayed for acrylamide analysis. Classical ODS-2 analytical columns showed poor separation since acrylamide co-eluted with the chromatographic front. In contrast, the ODS-2 column Synergi Hydro-RP showed an excessive retention for acrylamide (12 min). Because of that, different ODS-1 columns (partially deactivated) were assayed, founding excellent acrylamide separation (retention times ranged from 6 to 8 min).

The first mobile phase assayed was the one used by Zyzak et al. (2003) composed of 10 mM amonium acetate (pH 6) with 0.1% of each acetic and formic acid. This mobile phase was compared with a second mobile phase composed of methanol–water (1:99) added with formic or acetic acid. Mobile phase containing acetic acid significantly improves the response of acrylamide (m/z 72.1 H^+) about 1.35-fold than formic acid, and 4.74 times higher than the mobile phase with ammonium acetate. Acetic acid was selected as acidic modifier.

In order to adjust the fragmentation level it was performed a FIA assay by injecting 20 μl of both acrylamide standard (1000 μg/l) and potato sample extract and changing the cone voltage from 25 to 125 V. The best relation fragmentation-response was found at 100 V.

The ions monitored for identification and quantification of both analyte and internal standard were $[C_3H_5NO]^+ = 72.1$ and $[^{13}C_3H_5NO]^+ = 75.1$, respectively (Fig. 2). Quantitation was performed by comparison with a calibration curve (25–1000 μg/l) for acrylamide and [$^{13}C_3$]-acrylamide, both with correlation coefficients of 0.9978 and 0.9998, respectively. In every assay labeled acrylamide content was taken into account to correct for acrylamide recovery.

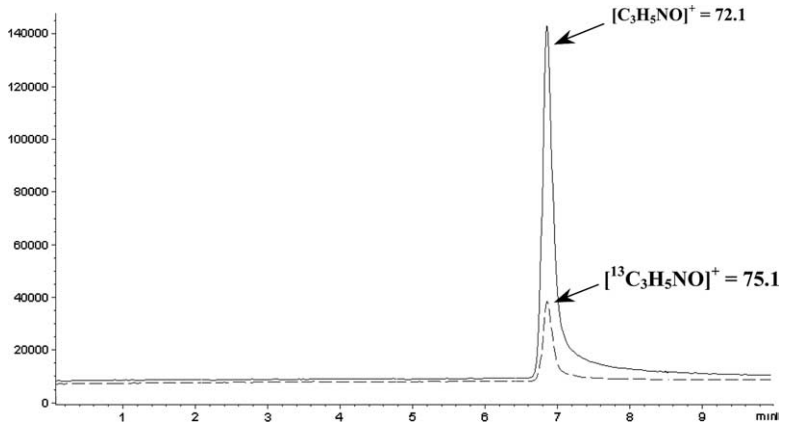

Fig. 2. Typical chromatogram profile of acrylamide (72.1 *m*/*z*) in a potato chip extract (1126 ± 44 μg/kg). Internal standard (75.1 *m*/*z*) concentration in sample (100 μg/kg).

3.4. Method performance

A control of analytical performance was implemented by the use of a reference material that was used as control in each series of analysis. The reference material was a potato chip sample simultaneously analyzed by Analycen (Lidköping, Sweden) in order to assess the reference value (575 ± 37 μg/kg).

A recovery assay (Table 3) was performed by adding 0.2, 0.4, 0.6, 0.8 and 1.0 mg of acrylamide/kg potato chip (each two independent determinations). It was observed a mean recovery of 98.8 ± 3.4% and a intra-day repeatability of 3.9%. A good coefficient of correlation of 0.9978 was achieved over the whole concentration range (200–1000 μg/kg).

The estimation of the detection limit (LoD) and the quantitation limit (LoQ) were performed according to Jezussek and Schieberle (2003) over the recovery assay. LoD is the addition value referring to the 95% confidence limit of the calibration line at the zero addition level. LoQ is the addition level that lowers the 95% confidence limit of the addition level at LoD. In this way the LoD and LoQ were of 23.2 and 91.8 μg/kg. If LoD and LoQ were extrapolated from the signal-to-noise (S/N) ratios obtained for the responses in the ion *m*/*z* 72.1, LoD could be similar but LoQ, taking an S/N ratio of 3:1, could be reduced until 69.6 μg/kg.

3.5. Sample analysis

Thirty-nine classical potato chips from 34 producers, without flavor or species added were analyzed for acrylamide content. Acrylamide content ranged from 211 to 5492 μg/kg with an average value of 1484 μg/kg (Table 4) and a median of 1180 μg/kg. A confidence interval (95%) from 1132 μg/kg to 1836 μg/kg was obtained. Similar values were obtained for other authors (Becalski et al., 2003; Roach et al., 2003; Rosen & Hellenäs, 2002; Presentation at HPLC, 2003; Tareke et al., 2002) who found acrylamide levels ranged between 224 and 3700 μg/kg. The consumption of potato chips is Spain was 1.6 kg/person/year, which gives a mean acrylamide intake of 6.5 μg/person/day; this is an important part of the daily intake of food-derived acrylamide and is of great importance since potato chips consumption is steadily expanding in Spain (a 28.4% increase since 1998) (Ministerio de Agricultura, 2003). In this way, the control of potato chips manufacture would be of interest to lower acrylamide formation and then to lower the mean acrylamide intake of the Spanish population. The Spanish Food National Agency (AESA) has recently started to estimate the incidence of acrylamide consumption by Spanish population in order to clarify the risk assessment for this chemical contaminant.

A more detailed study of sample distribution shows a high variability in the acrylamide content where several

Table 3
Recovery assay from potato chip reference material spiked with acrylamide[a]

Acrylamide added (μg/kg)	Theoretical acrylamide (μg/kg)	Measured acrylamide (μg/kg)	Mean recovery (%)
0	575	578 ± 13	100.6
200	775	749 ± 23	96.6
400	975	1007 ± 33	103.3
600	1175	1177 ± 11	100.1
800	1375	1363 ± 12	99.1
1000	1575	1471 ± 49	93.4

[a] Average values from two independent analysis.

Table 4
Statistical analysis of the acrylamide content (μg/kg) of different potato chip groups[a]

Group	Mean ± SD	Median	Maximum	Minimum	CI	*n*
Total	1484 ± 1086	1180	5492	211	1132–1836	39
Commercial	1543 ± 1144	1214	5492	219	1099–1986	28
Artisanal fried-potatoes	1335 ± 955	1056	3693	211	694–1977	11
Oily	1781 ± 1507	1377	5492	219	823–2738	12
Non oily	1298 ± 844	1073	4810	211	964–1632	27
Light-protected	1645 ± 1185	1191	5492	219	695–5492	24
Non light-protected	1225 ± 883	1106	3693	211	737–1715	15

[a] Average values from two independent analysis.

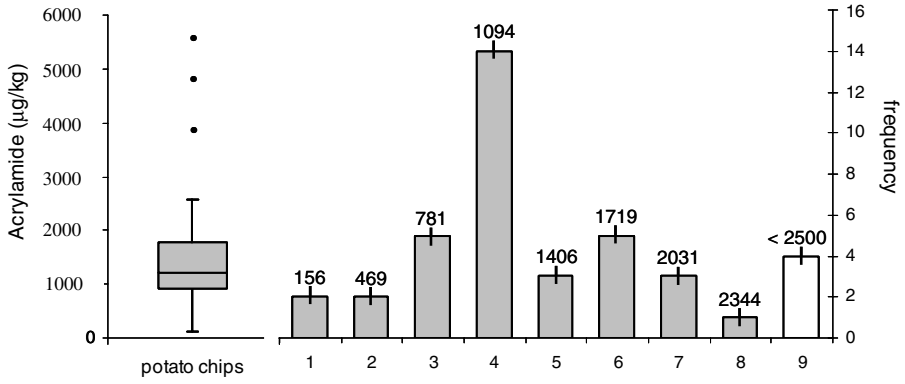

Fig. 3. Box-and-whisker plot (a) and frequency tabulation graph (b) from acrylamide content in Spanish potato chips. Midpoints are included for each analysis (see text for more detail).

samples are clearly over-processed (outliners from Fig. 3). A box-and-whisker plot was used since this graphical presentation uses a non-parametric test. In addition, to gain more insight on the acrylamide distribution a frequency tabulation graph was plotted. The samples were divided into 8 classes (interval range of 312.5 μg/kg) up to 2500 μg/kg and outliners were represented in group 9 (upper than 2500 μg/kg). The group with the highest frequency (29.5%) showed an acrylamide concentration ranged between 937.5 and 1250 μg/kg, and 10 % of the samples contain more than 2500 μg/kg. The high variability of the data is mainly a result of the variable raw material and processing conditions applied. Extent of the thermal treatments reveals as the main factor, and cooking oil (expressed as peroxide value) did not affect acrylamide levels, as well as soaking (Institute for Reference Materials & Measurements, 2004).

Samples were classified according to the type of processing (industrial vs. local fried shops); presence or not of oil drops on the container layer (oily vs. non-oily); and if the container was light-protected or not (Table 4). It was not observed statistically significant differences ($p < 0.05$) between commercial vs. artisanal fried-potato shops. The same differences were obtained for oily vs. non-oily potato chips and for **container-light-protected** potato chips vs. non-light-protected.

4. Conclusions

This work describes the determination of acrylamide in commercial potato chips by a confirmatory and quantitative analytical method. This procedure takes into account and evaluates different analytical approaches described previously in the literature (Ahn et al., 2002; Andrzejewski et al., 2004; Becalski et al., 2003; Biedermann et al., 2002; Mottram & Wedzicha, 2002). The sample clean up is based in the removal of co-extractives from the aqueous extracts by SPE cartridges. In addition, it must be underlined the importance of oil addition to avoid acrylamide losses during solvent removal, as described by Biedermann et al. (2002). The analyte is well retained on the LC column and the chromatographic step takes only 10 min per measurement. In addition, our lab participated in an international proficiency test organized by Institute for Reference Materials and Measurements (IRMM) on crisp bread, obtaining a good evaluation of our method (Riediker & Stadler, 2003). These data are useful to evaluate the dietary intake of acrylamide for the Spanish population for risk assessment purposes. At present, the procedure it has been evaluated in other food matrices to fullfil the acrylamide database in the Spanish diet.

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