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ORIGINAL PAPER

Determination of α-chaconine and α-solanine in commercial potato crisps by QuEChERS extraction and UPLC-MS/MS

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A simple and fast analytical method for the determination of the main steroidal glycoalkaloids, α-chaconine and α-solanine, in commercial potato crisps, based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) extraction and ultra performance liquid chromatography coupled with an electrospray ionization triple-quadrupole tandem mass spectrometer (UPLC-MS/MS) in the multiple reaction monitoring mode was established and validated. The sample preparation procedure involves the extraction of the analytes with acidified acetonitrile and simultaneous liquid–liquid partitioning achieved by an addition of anhydrous magnesium sulfate and sodium acetate without any further clean-up steps. The limits of quantification (LOQs) for α-chaconine and α-solanine were 31 μg kg^{-1} and 16 μg kg^{-1} of fresh mass, respectively, at the signal-to-noise ratio (S/Ns) of 10. The method was applied in a survey of the content of α-chaconine and α-solanine in twenty commercial potato crisps from different brands. The results showed that all the products contained α-chaconine and α-solanine in widely varying concentrations. The amount of α-chaconine was higher than that of α-solanine in all samples.

Keywords: steroidal glycoalkaloids, potato crisps, α-chaconine, α-solanine, QuEChERS, UPLC-MS/MS

Introduction

Potato crisps are popular snack food. Due to their generically pleasurable taste and texture, they are appreciated by consumers all over the world, especially by the younger population. Together with other food safety concerns regarding potato crisps, the content of steroidal glycoalkaloids (GAs) in these industrially prepared foodstuffs should not be neglected (Pęksa et al., 2006). GAs are secondary plant metabolites which are known to be highly toxic to humans and animals. It has been reported that low doses of GAs intake can cause gastrointestinal disturbances such as vomiting, diarrhea and abdominal pain (Hellenäs et al., 1992); while at higher doses, the toxicity of GAs leads to acute intoxication and more severe symptoms, including rapid pulse, low blood pressure, neurological disorders and, in severe cases, coma and death (Langkilde et al., 2009). GAs are studied mainly because of their impact on health through the consumption of food (Milner et al., 2011); in addition, the toxicants reportedly may accumulate in case of daily consumption (Mensinga et al., 2005) and thus pose a cumulative safety risk.

The most important GAs in potatoes are α-chaconine and α-solanine, both functioning as natural pesticides protecting the plant from fungi, insects, bacteria, etc. (Boulogne et al., 2012). These two naturally occurring toxins account for 95 % of the total GAs content in potatoes (Smith et al., 1996). The GAs level in a potato tuber is usually higher in the peel than in the tuber flesh (Nema et al., 2008);

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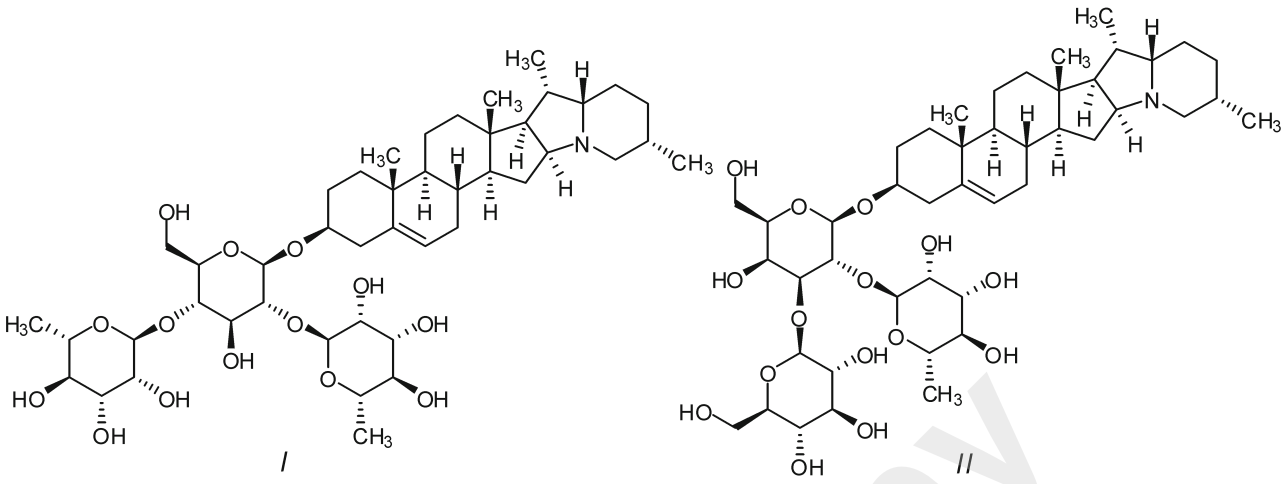

Fig. 1. Molecular structure of α-chaconine (*I*) and α-solanine (*II*).

also, mechanical damage or exposure to light can increase the GAs concentration (Machado et al., 2007; Mondy et al., 1987). Particularly high concentrations are found in green and sprouted tubers, especially around potato eyes (Friedman et al., 1997). Preliminary processing, such as peeling, can reduce these compounds significantly (Tajner-Czopek et al., 2008) but considerable amounts of GAs can still be detected in processed potato products; for instance, fried chips and other potato foodstuffs, even when home processing (boiling, frying, microwaving) methods are employed (Bushway & Ponnampalam, 1981; Lachman et al., 2013). Since they are one of the major contributors to daily GAs intake, particularly for children and teenagers, a survey on the GAs content in commercial potato crisps should be conducted, and reliable analytical methods have to be identified.

A number of methods for the determination of GAs in either raw or processed potato products have been developed, including thin layer chromatography (Bodart et al., 2000; Simonovska & Vovk, 2000), enzyme-linked immunosorbent assay (ELISA) (Friedman et al., 1998; Sporns et al., 1996), capillary electrophoresis (CE) (Bianco et al., 2003; Driedger et al., 2000), and high-performance liquid chromatography with an ultraviolet detector (HPLC-UV) (Maurya et al., 2013; Shakya & Navarre, 2006) or a tandem mass spectrometry detector (LC-MS/MS) (Sheridan & Kemnah, 2010; Zywicki et al., 2005). For the sample extraction and purification steps, the most popular technique is solid-phase extraction (SPE) (Distl & Wink, 2009; Rytel et al., 2005; Tömösközi-Farkas et al., 2006); however, this method is usually time-consuming and cost-intensive, and is therefore underutilized in bulk tests such as risk assessment and quality control in commercial production.

The QuEChERS sample preparation method introduced by Anastassiades et al. (2003) involves the extraction of target compounds from a homogenized sample using acetonitrile and salt in a centrifuge tube, followed by a solid-phase dispersive clean-up step performed in another test tube containing sorbents to remove interfering components. QuEChERS has many advantages, including the elimination of laborious steps and the subsequent need for automation. This technique also provides high sample throughput while simultaneously limiting solvent usage. The application of QuEChERS in food analysis involves pesticide residues (Sampaio et al., 2013), veterinary drug residues (Pereira Lopes et al., 2012), mycotoxins (Ferreira et al., 2012), etc. However, to the best of our knowledge, literature on the application of the QuEChERS methodology in the extraction of GAs from commercial potato crisps is scarce. In the present study, an analytical procedure based on UPLC-MS/MS with modified QuEChERS extraction was used for the determination of α-chaconine and α-solanine in commercial potato crisp. This method can be generally applied for research and risk assessment purposes.

Experimental

Reagents and materials

α-Solanine (purity $\geq$ 95 %) was purchased from Sigma–Aldrich (Shanghai, China); α-chaconine was purchased from ChromaDex (Irvine, CA, USA) (Fig. 1). HPLC grade acetonitrile (MeCN), methanol (MeOH), formic acid (HCOOH), and ammonium formate (AMF) were purchased from Dikma Technologies Inc. (Lake Forest, CA, USA). Analytical grade ammonium hydroxide (NH_4OH) was obtained from Beijing Chemical Plant (Beijing, China). Ultra-pure quality water which was generated in the laboratory by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout the experiments. Anhydrous magnesium sulfate ($MgSO_4$), anhydrous

Table 1. MRM transitions of α-solanine and α-chaconine with optimized parameters

Analyte	Parent ion (m/z)	Cone voltage/V	Daughter ion (m/z)	Collision energy/eV
α-Solanine	868.9	95	98.3 398.6	73 81
α-Chaconine	852.7	100	97.7 398.4	87 73

sodium acetate (NaOAc), sodium chloride (NaCl), sodium citrate dibasic sesquihydrate, and sodium citrate dehydrate were obtained from Dikma Technologies Inc. (Lake Forest, CA, USA). The sorbents used for dispersive solid phase extraction (d-SPE), including primary secondary amine (PSA) and C18 (particle size of 40–60 μm), were purchased from Dikma Technologies Inc. (Lake Forest, CA, USA).

Sample collection, pre-treatment and preparation

Twenty industrially prepared potato crisps of different brands were purchased from local supermarkets and retail outlets. All snack samples were packaged in rigid tubes or plastic bags, and labeled as containing potatoes or potato starch on the packages.

The samples were ground thoroughly in an A11 basic Analytical mill (IKA Works, Guangzhou, China), and the homogenized samples were stored at –20 ℃ in polyethylene screw containers.

The homogenized crisp samples (0.5 g each) were individually weighed and transferred to 50 mL Telfon centrifuge tubes containing 5 mL of water and they were vortexed for 1 min. Then, 25 mL of MeCN with 1 vol. % HCOOH were added to the tube, and the solutions were vortexed for 3 min. Afterwards, 2 g of $MgSO_4$ and 1 g of NaOAc were added, the solution was shaken vigorously by hand for 30 s and then vortexed for another minute to separate the water and the MeCN layers. The tube was subsequently centrifuged at 4 ℃ and 9000 min^{-1} for 8 min. A 0.5 mL aliquot of the supernatant was pipetted into a new 15 mL centrifuge tube and evaporated to dryness under nitrogen atmosphere at 40 ℃ (water bath). The dry residue in the tube was redissolved in 10 mL of the solvent (MeCN/water, $\varphi_r = 1 : 1$) and an 1 mL aliquot of the solution was filtered through a 0.22 μm filter membrane for the UPLC-MS/MS analysis.

Instrumentation

Chromatographic separation was performed on an ACQUITY^{TM} Ultra Performance LC system (Waters Corp., Milford, MA, USA) with a cooling autosampler and a column oven. An Endeavorsil^{TM} UHPLC C18 column (100 mm × 2.1 mm, 1.8 μm; Dikma Technologies Inc., Lake Forest, CA, USA) was employed for the separation; the column temperature was maintained at 40 ℃. The mobile phase was composed of a 10 mmol L^{-1} AMF solution (A) and MeCN (B). The gradient elution program, with the constant flow rate of 0.25 mL min^{-1}, started with solution A at 95 % for 0.2 min, followed by a linear decrease to 5 % in 3 min, held for 1 min, and then increased to 95 % in 0.5 min, and held for another 2 min as column equilibration. The total operation time was 8 min. The autosampler was conditioned at 10 ℃ and 5 μL of the sample solution were injected.

A triple-quadrupole tandem mass spectrometer (Micromass® Quattro Premier XE mass spectrometer, Waters Corp., Milford, MA, USA) equipped with an electrospray ionization (ESI) interface was used for analytical detection. The ESI source was operated in the positive ionization mode (ESI+). The optimal MS parameters were as follows: capillary voltage of 3.0 kV, source temperature of 100 ℃ and desolvation temperature of 450 ℃. High-purity nitrogen was used as the desolvation and cone gas with the flow rate of 600 L h^{-1} and 50 L h^{-1}, respectively. Quantification was performed in the mode of multiple reaction monitoring (MRM), and the optimized MRM transitions, cone voltages and collision energy voltages for α-chaconine and α-solanine were determined (Table 1). The dwell time of each ion pair in the mixture solution was 50 ms. All data were acquired by the MassLynx^{TM} NT 4.1 software and processed using the QuanLynx^{TM} program (Waters Corp., Milford, MA, USA).

Results and discussion

Optimization of UPLC-MS/MS conditions

The MS parameters were optimized by flow injection analysis, and the MRM transitions per analyte were individually monitored and chosen in the ESI positive mode at the optimum conditions using the "AutoTune" of the Mass Tune program; subsequently, they were confirmed by an injection of the standard solution of analytes at proper concentrations. Table 1 shows the specific conditions for each compound.

To obtain the lowest detection limit for the GAs compounds, the chromatographic conditions were studied using a standard solution (10 ng mL^{-1}) to obtain optimal peak shape and maximum responses on the Endeavorsil^{TM} C18 column. Because mobile

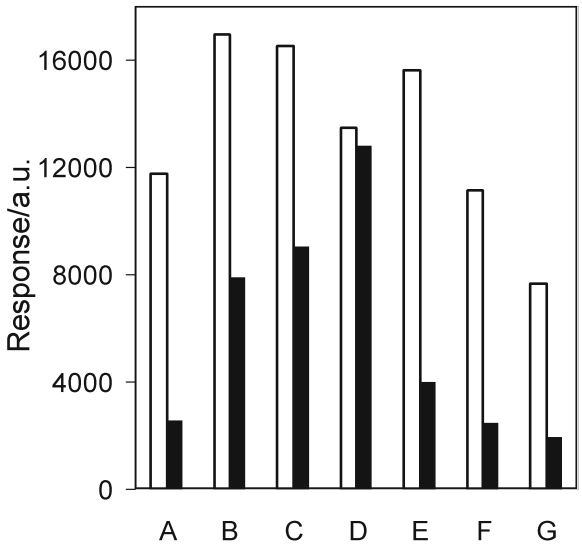

Fig. 2. UPLC-MS/MS responses of α-chaconine (□) and α-solanine (■) for different mobile phases: water/MeCN (A); 0.1 vol. % HCOOH in water/MeCN (B); 1 mmol L^{-1} AMF/MeCN (C); 0.1 vol. % HCOOH in water and 1 mmol L^{-1} AMF/MeCN (D); 0.1 vol. % HCOOH in water/MeOH (E); 1 mmol L^{-1} AMF/MeOH (F); 0.1 vol. % HCOOH in water and 1 mmol/L AMF/MeOH (G).

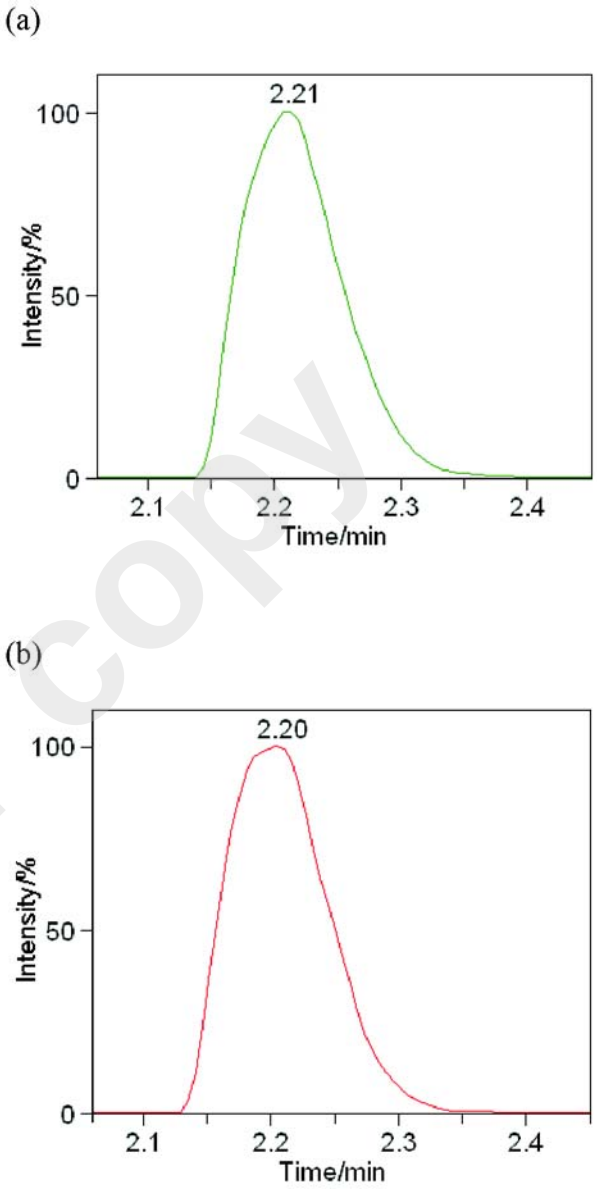

Fig. 3. Typical chromatogram of α-chaconine (a) and α-solanine (b) from a crisp sample.

phases have a significant impact on the ionization of the target compounds, several mobile-phase compositions were studied. Both MeCN and MeOH were tested as the organic phase. A solution with 0.1 vol. % HCOOH, 1 mmol L^{-1} AMF and buffer (0.1 vol. % HCOOH and 1 mmol L^{-1} AMF) was tested as the aqueous mobile phase. In this experiment, the mobile-phases composed of the MeCN and buffer solutions provided better chromatographic conditions and stronger responses than other combinations (Fig. 2). In addition, the gradient was optimized to obtain good peak shape and elute the analytes within 8 min, including the cleaning and re-equilibration steps. It is necessary to clarify that the two GA compounds were difficult to separate on a UPLC column within 8 min due to their very similar retention behavior; however, MS/MS detection obviated the need for extremely significant separation. Moreover, no interfering peak was observed when separately injecting the analytes and monitoring the response in the other channel. Fig. 3 shows the typical chromatogram of α-chaconine and α-solanine from a crisp sample.

Optimization of QuEChERS

The original QuEChERS consists of two steps: salting-out extraction and partitioning, followed by a d-SPE clean-up. In the extraction step, several extractant solvents were tested, including MeCN, MeOH, acidified MeCN (1 vol. % HCOOH), and acidified MeOH (1 vol. % HCOOH). Since MeCN is recommended as the extractant solvent by the official QuEChERS method, two salt combinations (AOAC and EN) (AOAC International, 2007; CEN, 2008) were added to MeCN and acidified MeCN for the partitioning of the analytes from food samples into an organic layer. As shown in Fig. 4, relatively high responses of α-chaconine and α-solanine were observed when acidified MeCN with the AOAC salt ($MgSO_4$ and NaOAc) was employed; so, this extraction procedure was used in all further experiments.

Additionally, acidity of the extractant solvents was evaluated by adding different amounts of HCOOH (vol. %: 0, 0.5, 1, 2.5, 5) to MeCN. As shown in Fig. 5, 0.5 vol. % HCOOH in MeCN gave the highest response of α-solanine, whereas 1 vol. % HCOOH in MeCN gave

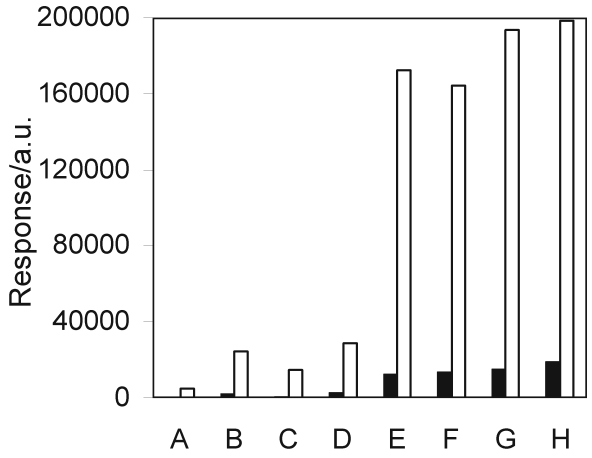

Fig. 4. UPLC-MS/MS responses of α-chaconine (□) and α-solanine (■) for different QuEChERS extraction procedures: 25 mL of MeCN (A); 25 mL of MeOH (B); 0.1 vol. % HCOOH in MeCN (C); 0.1 vol. % HCOOH in MeOH (D); MeCN/EN salt (E); MeCN/AOAC salt (F); 0.1 vol. % HCOOH in MeCN/EN salt (G); 0.1 vol. % HCOOH in MeCN/AOAC salt (H).

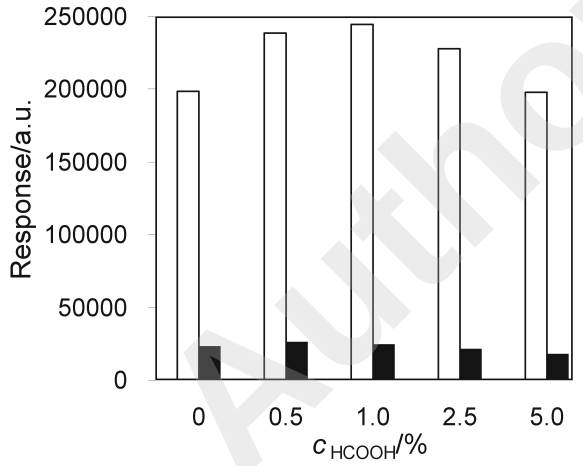

Fig. 5. UPLC-MS/MS responses of α-chaconine (□) and α-solanine (■) for different HCOOH content (vol. %) in the extractant solvents.

the highest response of α-chaconine; 1 vol. % HCOOH was chosen due to the solvent economy.

A clean-up procedure based on d-SPE was also evaluated. Various clean-up materials have different purpose. For example, C18 is used to remove long chain fatty compounds, sterols, and other non-polar interfering compounds from the extracting solution, whereas PSA are used to remove fatty acids, sugars, organic acids, and pigments. In this work, three clean-up steps were tested including: (a) 50 mg of PSA and 150 mg of anhydrous $MgSO_4$; (b) 50 mg of C18 and 150 mg of anhydrous $MgSO_4$; (c) 50 mg of PSA, 50 mg of C18, and 150 mg of anhydrous $MgSO_4$. Unfortunately, none of these materials provided promising results compared with the procedure without the d-SPE steps. These outcomes support previous reports on the limited usability of clean-up procedures (Jandrić et al., 2011) and therefore, no clean-up procedure was employed in this method.

Method validation

Method validation was based on the evaluation of linearity, limits of quantification (LOQs), accuracy, and precision.

To compensate the matrix effects, matrix-matched standard calibration curves were used for the quantification of α-chaconine and α-solanine in crisp samples. Stock solutions containing two analytes were diluted to a series of appropriate concentrations (ng mL^{-1}: 10, 25, 50, 75, 100) with the sample extract solution. Aliquots of the diluted solutions were evaporated to dryness under nitrogen in a 40 ℃ water bath and redissolved in the injection solvent (MeCN/water, φ_r = 1 : 1). The standard solution series were injected, in triplicate, into UHPLC-MS/MS to obtain the calibration curves. Both curves showed good linearity with the coefficient of correlation (r^2) exceeding 0.9993. Detailed descriptions of the regression curves are summarized in Table 2.

LOQs of the proposed method for α-chaconine and α-solanine were calculated from the signal-to-noise ratios of the individual quantitative ion peaks, assuming the minimum detectable signal-to-noise level of ten. LOQs for α-chaconine and α-solanine were 31 μg kg^{-1} and 16 μg kg^{-1} fresh mass, respectively, both of which are much lower than the levels of GAs in commercial crisps, indicating thus that the methodology exhibits excellent sensitivity for the quantification of these two compounds (Table 2).

Recovery and reproducibility of the method were determined in order to evaluate its accuracy and precision, respectively. Three different concentrations of the standard solution (low, medium, and high) were

Table 2. Regression curves, calibration range (RG_{calib}), correlation coefficients (r^2), and LOQs of α-chaconine and α-solanine

Analyte	Regression curve	RG_{calib}/(ng mL^{-1})	r^2	LOQ/(μg kg^{-1})[a]
α-Chaconine	$y = 6011.21x + 2736.34$	10–100	0.9993	31
α-Solanine	$y = 1235.78x + 877.64$	10–100	0.9996	16

a) μg per kg of fresh mass.

Table 3. Average recoveries ($R_{average}$) and RSD at three spiked levels of α-chaconine and α-solanine ($n = 5$)

Analyte	Matrix/(ng mL^{-1})	Detected/(ng mL^{-1})	Added/(ng mL^{-1})	$R_{average}$/%	RSD/%
	23.4	30.9	10.0	75.0	9.2
α-Chaconine		61.0	50.0	75.2	8.9
		104.2	100.0	80.8	9.8
	13.2	20.5	10.0	73.0	12.2
α-Solanine		51.2	50.0	76.0	8.5
		96.8	100.0	83.6	6.1

added to the control sample, and average recoveries ($R_{average}$) of the analytes were obtained. The recovery (R) was calculated as $R = (C_{detected} - C_{matrix})/C_{added} \times 100$ %, where $C_{detected}$ is the concentration in a spiked sample, C_{matrix} is the concentration in the control sample prior to spiking and C_{added} is the concentration of addition. The $R_{average}$ values of α-chaconine and α-solanine with three spiked levels are presented in Table 3. Precision of the method (expressed as the relative standard deviation, % RSD) was evaluated by a five-fold analysis of the crisp samples and the spiked samples. As shown in Table 3, RSD values at the three spiked levels considered were in the range of 6.1–12.2 %.

Application to commercial potato crisp samples

The method was applied to twenty samples of commercial potato crisps purchased from local supermarkets. All samples claimed to contain either potato slices or potato starch according to their packaging information, and they were all found to be GAs-positive at various concentrations. The concentration of GAs found in these samples ranged from 13.7 mg kg^{-1} to 46.6 mg kg^{-1} for α-chaconine, and from 2.7 mg kg^{-1} to 13.9 mg kg^{-1} for α-solanine. The α-chaconine/α-solanine ratio also varied for individual samples; however, the concentration of α-chaconine was always higher than that of α-solanine, which is in accordance with the found in raw potatoes or potatoes processed by other cooking methods, indicating that industrial crisps preparation cannot eliminate α-chaconine or α-solanine from the products.

Conclusions

A simple analytical method based on QuEChERS extraction and UPLC-MS/MS detection for the determination of α-chaconine and α-solanine in commercial potato crisps was presented. The sample preparation procedure employed in this method is fast and cost-effective. The method was validated and applied in real sample analysis, where it was shown to be successful in the measurement of the two steroidal glycoalkaloid compounds in potato crisps.

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