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Optimization of QuEChERS sample preparation method for acrylamide level determination in coffee and coffee substitutes

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ABSTRACT

Modification of QuEChERS sample preparation method for the determination of acrylamide (2-propeneamide, AA) in coffee (roasted and instant) and coffee substitutes via silylation has been reported. The AA level after conversion to *N,O*-bis-(trimethylsilyl)acrylamide (BTMSA) was determined with gas chromatography/ion-trap mass spectrometry in a single ion monitoring mode (GC-SIM-MS). A sample of instant coffee was used to develop an analytical method in the optimisation experiment, which involved the selection of the most suitable sorbents for the dispersive solid phase extraction (d-SPE) clean-up. The usefulness of the method has been verified based on the recovery ratio of acrylamide (fortified samples analysis). Seven variants of the method were tested. The obtained results showed that the best recovery of 95% was obtained using a combination of PSA and SAX sorbents with the addition of hexane in the initial step of extraction. The chosen method was applied for the AA determination in 17 roasted, 10 instant and 8 coffee substitutes samples. The results of acrylamide content ranged from 17.7 ± 1.4 to 776.1 ± 8.8 μg kg^{-1} in roasted coffee, from 96.4 ± 1.7 to 346.5 ± 10.9 μg kg^{-1} in the case of instant coffee, and finally from 70.0 ± 6.0 to 188.9 ± 0.1 μg kg^{-1} in the samples of coffee substitutes. The obtained results are in good agreement with previous literature reports.

1. Introduction

Risk factors in food are either from chemical or microbiological origin, or a combination of both of them. Some of the major groups of chemicals risk factors, except toxins from bacteria, are inter alia compounds formed during processing of food products. In addition, a range of compounds is formed in the temperature-dependent Maillard reaction. Among them we can mention heterocyclic amines (HCA) [1], furan [2], hydroxymethylfurfural (HMF) [3], lysinoalanine (LAL) and compound of our interest – 2-propeneamide. Acrylamide is a compound with a potential to cause a spectrum of toxic effects, including neurotoxic effects as it has been observed in humans [4]. It has also been classified as a "potential human carcinogen" [5]. The mutagenic and carcinogenic properties of AA are assumed to depend on the epoxy metabolite, glicydamide [6]. Several AA formation mechanisms were published in available literature [7,8]. Amino acid – asparagine and compounds with carbonyl group, especially reducing sugars like glucose and fructose, are used as AA precursors. Foods rich in both of aforementioned compounds are largely derived from plant sources such as potatoes, cereals (barley, rice, wheat) and coffee. Therefore, acrylamide arises from these ingredients at the temperature above 120 °C [9]. Coffee and coffee substitutes are some of the most popular beverages in the world. They are prepared from roasted cereals or vegetables. The crucial step for its production is roasting. This process allows developing color, aroma and flavour, which are essential and specific for coffee and coffee substitutes [10]. Unfortunately, the same processes lead to the formation of acrylamide. So far, the AA levels have been usually studied in roasted and instant coffees, but to our best knowledge, information about acrylamide content determination in coffee substitutes is still limited.

Many analytical methods for acrylamide identification and quantification have been reported since the announcement of its findings in 2002. However, up till now an official method of AA quantification using GC has still not been established. Although there are three official AA determination methods but involving only LC–MS/MS [11], on which, unfortunately not everyone can afford. Due to its high solubility in water, high reactivity and lack of chromophor, AA is not easy to detect. Chromatographic methods with UV detection are not selective enough for its determination at its low level. Therefore, the majority of applied methods have been based on gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled with a mass spectrometer [12]. In contrast to the AA determination method involving LC/MS [13] in the case of GC method, derivatization process is needed. As derivatization agents 2,3-dibromopropionamide [14], *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) [15] and trifluoroacetic anhydride (TFAA) [16] have been usually applied. The GC–MS application for AA determination in coffee and coffee products was developed

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by Soares et al. [17] and later applied by Alves et al. [18] to espresso coffee and again by Soares et al. [19]. In the above-mentioned method matrix solid-phase dispersion (MSPD) was used. Solid-phase micro extraction (SPME) was also applied in other research [20]. Nevertheless, for the AA determination in food products the extraction with water, aqueous solution of acetonitrile with methanol, and ethyl acetate were the most frequently used sample treatment methods [12]. Also, the extracted acrylamide amount using Soxhlet extraction was proposed [21]. However, the main drawback is the long extraction time. To overcome these problem Brandl et al. [22] have extracted acrylamide selectively by using accelerated solvent extraction (ASE). Most clean-up procedures consisted of the combination of several solid-phase extractions (SPE), especially three different SPE cartridges combination [23, 24]. However, analysis of more difficult food matrices, such as coffee and coffee substitutes is one of the major pitfalls in the acrylamide determination [11].

Wherefore looking for effective complex samples technique for the sample preparation for AA determination we decided to implement the QuEChERS approach, which consists of two steps: extraction with organic solvent and dispersive solid-phase extraction (d-SPE) for extract clean-up. This sample treatment had been originally developed by Anestassiades et al. [25] for plan origin sample preparation for pesticides residues determination. Nonetheless, to our best knowledge, the usefulness of QuEChERS concept for sample preparation in the AA determination has still remained unexplored. Additionally, modified d-SPE and silylation to *N,O*-bis-(trimethylsilyl)acrylamide were introduced for coffee and coffee substitutes samples preparation for the very first time. The outcomes from our previous research concerning coffee samples [26] led to select the most suitable sorbent to this food matrix. Thus, the aim of this study was to evaluate the use of the modified QuEChERS method for the acrylamide determination in coffee and coffee substitutes via silylation with GC-SIM-MS detection. Moreover, the previously optimized procedure was used to assess the level of AA contamination in roasted, instant and coffee surrogates purchased at Polish retail market.

2. Materials and methods

2.1. Chemicals

Acrylamide purum ≥98% (GC), acrylamide-d_3 standard solution for food analysis and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were obtained from Sigma-Aldrich Chemie GmbH, Germany. Magnesium sulphate anhydrous p.a. and sodium chloride p.a., were purchased from POCh SA, Poland. Acetonitrile and hexane, HPLC grade for liquid chromatography LiChrosolve® was purchased from Merck KGaA, Germany. PSA (primary and secondary amine), SAX (strong ion exchange), C_{18} (octadecylsilane), FL (florisil) and ENV (styrene-divinylbenzene) SPE Bulk Sorbent derived from Agilent Technologies, USA. Stock, intermediate and working standard solutions of AA at a concentration of 1 μg mL^{-1}, d_3-acrylamide (IS) at 5 μg mL^{-1} were prepared in acetonitrile.

2.2. Equipment

The Varian 4000 GC/MS (Varian, Inc., USA) system consisting of a 3800 GC with a CP-8410 auto-injector (Bruker, USA) and a 4000 Ion Trap MS detector was applied in chromatographic assays. The injector was a CP-1177 Split/Splitless Capillary Injector, with a temperature of 250 °C, and an injection volume of 1.0 μL. Each injection was performed in triplicate. Chromatographic separations were performed on a DB-5MS column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies, USA). The GC oven was operated with the following temperature program: initial temperature 50 °C – 3 °C min^{-1} – 100 °C – 25 °C min^{-1} – 250 °C (5.0 min). The analyses were performed with a solvent delay of 8.0 min. Helium 5.0 (Linde Gas, Poland) was used as the GC carrier gas at a flow rate of 1.0 mL min^{-1}. The ion trap mass spectrometer was operated in the internal ionisation mode, scan *m/z* from 45 to 500. The emission current of the ionisation filament was set at 15 μA. The trap and the transfer line temperatures were set at 180 and 220 °C respectively. Analyses were carried out in the selected ion monitoring mode (SIM) based on the use of one quantitative ion of BSTFA derivatives (128.1 for BTMSA, 132.1 for BTMSA-d_3), qualitative ions (128.2, 129.2, 131.1 for BTMSA, 132.2, 204.2, 220.1 for BTMSA-d_3) and retention times (8.81, 8.65 for BTMSA and BTMSA-d_3, respectively). Acquisition and processing data were performed using Varian Start Workstation software and NIST 2.0 library. MS1 Minishaker (IKA, Germany), MPW 350 R Centrifuge (MPW Med. Instruments, Poland) were employed during the sample preparation. Accublock™ (Labnet, USA) with nitrogen 5.0 (Linde Gas, Poland) was used to evaporate the solvent, and concentrate the extracts.

The BFTSA derivatives of AA and AA-d_3 were identified by comparing the retention time and quantitative and qualitative ions with the NIST library. Calibration curve was constructed by plotting the ratio of the peak area, divided by the peak area of the internal standard, against concentration of the analyte.

2.3. QuEChERS sample preparation method development

The sample of one instant coffee was used to develop the analytical method in the first part of the study – optimisation experiment. For this purpose series of experiments were conducted, which covers the selection of appropriate, additional sorbent for clean-up of the samples (C_{18}, SAX, florisil or ENV), apart from PSA sorbent, which is commonly used in QuEChERS method. Besides of acetonitrile, hexane (hx) was also added in the extraction step to selected samples. Seven variants of the method, shown in Table 1, were tested. Because until this time coffee and related food matrices with certified content of acrylamide are not commonly available, the usefulness of the method was verified on the basis of the recovery ratio of analysed compounds.

1 g of the samples was weighted into a 50-mL centrifuge tube, spiked with AA and AA-d_3 solutions to the fortification level of 0.05 mg kg^{-1}, mix and left to stand for 15 min at a room temperature prior to extraction. Then, 5 mL of hot, boiled water was added to the sample. After cooling to room temperature, 10 mL of acetonitrile (MeCN) with or without hexane (see Table 1), was added. The whole tube was vigorously shaken for 1 min, after which 1 g of NaCl and 4 g of $MgSO_4$ were added. This was followed by shaking for 1 min, and the solution was finally centrifuged for 15 min at 8700 RCF. 6 mL of the supernatant was placed in a PP 15 mL tube containing appropriate combination of sorbents (according to the scheme in Table 1) and 0.90 g of $MgSO_4$. After 1 min of shaking and 5 min of centrifuging at 5000 RCF, 4 mL of supernatant was transferred into a 4 mL screw cup vial and evaporated to dryness under a stream of N_2. The residues were dissolved in 500 μL of MeCN. 100 μL of the extract were placed in 1.5 mL screw cup vial containing 50 μL of BSTFA and were placed in a preheated, aluminium inset of Accublock™. The silylation reaction of AA to BTMSA was conducted for 1 h in 70 °C. After cooling, 200 μL of hexane were added

Table 1
Schema of experiment conducted in the study.

Tested factors		Chemicals	Quantity	Variant of the method						
				1	2	3	4	5	6	7
Solvent:	Extraction:	MeCN	10 mL	+	+	+	+	+	+	+
	Additional:	Hx	5 mL	−	−	−	−	+	+	+
Sorbent:	Basic:	PSA	0.15 mg	+	+	+	+	+	+	+
	Additional:	SAX	0.30 mg	+	+	+	+	+	+	+
		C_{18}	0.15 mg	−	+	−	−	−	−	−
		Florisil	0.15 mg	−	−	+	−	−	+	−
		ENV	0.15 mg	−	−	−	+	−	−	+

PSA – primary and secondary amine, SAX – strong ion exchange, C_{18} – octadecylsilane, FL – florisil, ENV – styrene-divinylbenzene SPE Bulk Sorbent, MeCN – acetonitrile, Hx – hexane.