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Acrylamide Content of Turkish Black Tea, Instant and Turkish Coffee Samples

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Abstract: Acrylamide contents of Turkish and instant coffee and black tea samples were determined by the gas chromatography mass spectrometry without any derivatization step in this present study. The sample preparation method included two primary clean up steps. In the first step, ethanol and solutions of Carrez I and II were added into sample mixtures. The second step with solid-phase extraction C18 cartridge was carried out for clean-up. The limit of detection and limit of quantification were 1.11 and 3.66 ng/mL, respectively. The quantification level of acrylamide was determined in eight instant coffee samples in the range between 4.48 and 15.71 µg/kg while seven Turkish coffee samples had a high content of acrylamide ranging from 3.89 to 88.44 µg/kg. Acrylamide was detected in only five samples from seventeen black tea samples at a quantification level between 7.02 and 19.85 µg/kg.

Türk Siyah Çayı, Hazır Kahve ve Türk Kahvesi Örneklerinin Akrilamid İçeriği

Anahtar Kelimeler
Türk kahvesi,
Hazır kahve,
Siyah çay,
Akrilamid,
SPE,
GC-MS

Özet: Bu çalışmada, Türk ve hazır kahve ile siyah çay örneklerinin akrilamid içerikleri, herhangi bir türevlendirme basamağı olmadan gaz kromatografisi kütle spektrometrisi ile belirlenmiştir. Numune hazırlama yöntemi iki aşamadan oluşmaktadır. İlk basamak, izolasyon aşamasıdır. Bu adımda, etanol ile Carrez I ve II çözeltileri örnek karışımlarına eklenmiştir. Katı faz ekstraksiyonu ikinci adımı oluşturmaktadır. Çalışmada, C18 kartuşu temizleme için kullanılmıştır. Tespit ve tayin limiti sırasıyla 1,11 ve 3,66 ng/mL'dir. Sekiz hazır kahve örneğinde 4,48 ile 15,71 µg/kg arasında değişen akrilamid miktarı belirlenmiştir. Yedi Türk kahvesi numunesi ise, 3,89 ile 88,44 µg/kg arasında değişen yüksek bir akrilamid içeriğine sahiptir. On yedi siyah çay numunesinden sadece beş tanesinde, 7,02 ile 19,85 µg/kg arasında değişen miktarda akrilamid tespit edilmiştir.

1. Introduction

Acrylamide is a chemical compound, which has been used for over fifty years as a monomer to synthesize polyacrylamides. Acrylamides are used as flocculants to purifying drinking water, and they used in many several industrial sectors such as paper, plastics, textile, dyes and cosmetics [1]. Acrylamide is primarily present in a variety of products such as caulking, food packaging, some adhesives, potatoes, bread, cacao powder, chocolate, chips, cookies and biscuits. Also it can form in coffee products during roasting processes of coffee beans [1, 2].

The presence of different acrylamide concentrations in foodstuffs was first reported in 2002 [3]. Results on the monitoring program of acrylamide levels in foodstuffs in Europe from 2007 to 2009 have shown the presence of that different concentration of acrylamide in various food products [4]. International Agency for Research on Cancer has classified acrylamide as probably carcinogenic to humans

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(Group 2A) [5]. Experimental studies on acrylamide exposure in animals have shown an increase of tumors in several central systems, thyroid, uterus, clitoral gland, oral tissue adrenal, pituitary, mammary glands etc. [6-11].

Methods for the analysis of acrylamide usually involve chromatographic separation followed by a spectrophotometric detector (UV, DAD), an electron capture detector (ECD) or mass spectrometry (LC-MS/MS, GC-MS, GC-MS/MS)) [12-14]. Mass spectroscopy analyses of acrylamide are mostly based on derivatization or without derivatization [2, 15-17]. Few procedures have been developed to remove the derivatization step and measure acrylamide directly after extraction and clean-up [18].

The aim of this present study is to determine the acrylamide contents of commercial black tea, instant coffee and Turkish coffee samples (n=61) without any derivation step.

2. Material and Method

1.1. Chemicals and reagents

Sep Pak Plus C18 cartridges of the Waters brand (Milford, MA, USA) were used for solid-phase extraction. Zinc sulfate heptahydrate (Carrez I), potassium hexacyanoferrate (II) trihydrate (Carrez II), acrylamide standard, methanol (HPLC grade) and acetone (GC grade) were obtained from Sigma Aldrich (St. Louis, MO, USA).

1.2. Coffee and tea samples

Commercial Turkish black tea (n=17), instant (n=27) and Turkish coffee (n=17) samples were purchased from local stores.

1.3. Sample isolation technique

The method reported by Şenyuva and Gökmen [2] was used for the isolation of acrylamide from samples. Each sample (5 g) was dissolved in a mixture of water (10 mL) and absolute ethanol (15 mL) by shaking vigorously for a minute and then the mixture was kept at -20°C for 15 min. Each mixture was centrifuged at 15,000 *g* for 5 min at 4°C. Supernatants were acidified with glacial acetic acid until pH reached about 4–5. Afterwards, Carrez I (1 mL) and Carrez II (1 mL) clearing solutions were added to the flasks, and then the mixture was shaken vigorously and kept at 4°C for 30 min. This solution was centrifuged at 15,000 *g* for 5 min at 4°C and supernatant was filtered through a 0.45 µm syringe nylon filter (Sartorius, Goettingen, Germany). Solvent was partly removed by a rotary evaporator (Heidolph, HL/HB G3) at 55°C, and then evaporator vessel was washed with 2 mL of water which was added to the solution in the vial.

1.4. SPE clean-up procedure

Sep Pak Plus C18 cartridges were placed in a manifold system and activated with 10 mL methanol and finally 10 mL rinsing water. The sample solution (5 mL) was loaded onto the column, and then sorbents were dried. Acrylamide was eluted from the cartridges using 2 mL acetone [19].

1.5. Calibration standard

Stock standard solution of acrylamide (10 mg/mL) was prepared in acetone, and six different concentrations were used for the calibration curve. Calibration curve was obtained by plotting the peak areas against the concentration of standard acrylamide solutions. The LOD value was defined as three times the background noise of the chromatographic instrument. The extraction recovery was determined by spiking samples with acrylamide in three replicates, and they were extracted as previously described.

1.6. Chromatography and apparatus

An Agilent 7890A gas chromatography unit equipped with a 5975 mass detector (MSD), a 7693B automatic sampler and a MSDCHEM (Agilent, Santa Clara, CA, USA) data system was used for the determination of acrylamide in coffee and tea samples. Analytes were separated in a fused silica capillary column DB-Wax. The carrier gas (helium) flow rate was 1 mL/min. Oven temperature program was as follows: initial temperature 60°C, held for a minute, increased to 240°C at 20°C/min, held at 20 min. The injection port, detector and ion source temperatures were 240, 250, and 230°C, respectively. The injection volume was 1 µL, and identification was determined using the selective ion monitoring (SIM) mode (m/z = 71) [20].

3. Results and Discussion

1.1. Method assessment

The calibration curve and linear regression analysis obtained by plotting the concentration of acrylamide (X) against the peak areas (Y) were used for the quantification of acrylamide in black tea, instant and Turkish coffee samples, and the limit of detection (LOD), limit of quantification (LOQ) and recovery values for the method are shown in Table 1. Liu et al. [21] evaluated the solid phase extraction method, and reported the LOD value of 1 ng/mL. Russo et al. [22] studied acrylamide in cereal-based foods and potato chips, and reported LOD value 2 ng/g.

Table 1. Analytical performance of acrylamide analyses in the studied matrices

Compound	Y	R^2	LOD (ng/mL)	LOQ (ng/mL)	Average recovery (%) and SD values
Acrylamide	913.12+153947X	0.999	1.11	3.66	Tea: 91.05 (0.95) Instant coffee: 93.12 (1.58) Turkish coffee 92.45 (0.98)

Y: regression equation; R^2: correlation coefficient; LOD: Limit of detection; LOQ: limit of quantification, SD: Standard deviation.

The percent recovery of acrylamide ranged from 91.05% to 93.12%, with RSDs less than 2.50% for sample isolation technique + SPE clean-up procedure. Soares et al. [19] evaluated the effectiveness of extraction method, and the recovery values were between 97.4 and 108.4%. Mizukami et al. [23] studied acrylamide in green tea, and recoveries varied from 94 to 108%.

1.2. Analytical results

Approximately 30% of black tea and instant coffee samples had acrylamide contents higher than the LOD value of the method while this ratio was about 40% for Turkish coffee samples (Table 2). On average, Turkish coffee samples contained three times more acrylamide than instant or black tea samples.

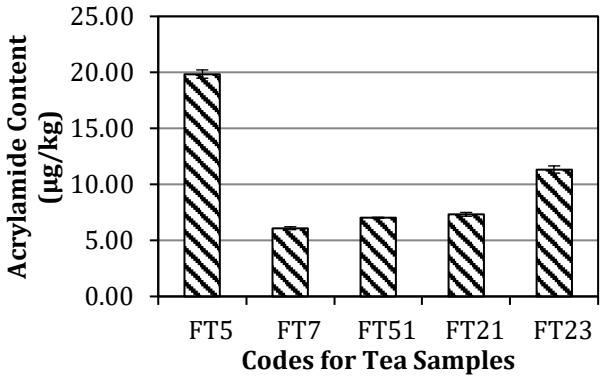

Figure 1. Acrylamide content in Turkish black tea samples (bars indicate standard deviations)

Five of black tea samples had an acrylamide level higher than LOD value of 2.37 µg/kg, and acrylamide content of these samples ranged from 6.09 to 19.85 µg/kg (Figure 1). Yoshida et al. [24] determined the acrylamide contents of tea samples and reported that roasted green tea samples had an acrylamide content ranging from 190 to 570 µg/kg while black tea samples had a level of 20 µg/kg. Acrylamide content ranged from 20 to 100 µg/kg in green tea samples including pan fired teas. Mizukami et al. [23] determined acrylamide contents in green tea samples by GC-MS method with a bromine derivatization step. They reported that the acrylamide contents ranged from 27 to 110 µg/kg in green tea, from 247 to 1880 µg/kg in roasted green tea and from 18 to 25 µg/kg in black tea samples. They reported that the acrylamide level in roasted tea products was controlled by asparagine in the presence of reducing sugars. Liu et al. [21] determined the levels of acrylamide in 30 tea samples less than 100 µg/kg, and reported that black, oolong, white and yellow tea samples had lower acrylamide contents (<20 µg/kg) than baked, roasted, and one sun-dried green tea samples (46–94 µg/kg). Relatively low acrylamide content of fresh tea samples is probably because tea leaves are not dried at high temperature while drying process at 100–150°C may be responsible for high acrylamide levels in baked, roasted, and one sun-dried green tea samples [21]. In our study, acrylamide levels in black tea samples were similar to these studies.

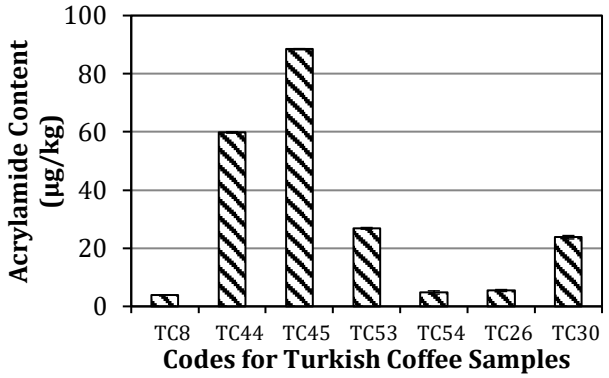

Figure 2. Acrylamide content in Turkish coffee samples (bars indicate standard deviations)

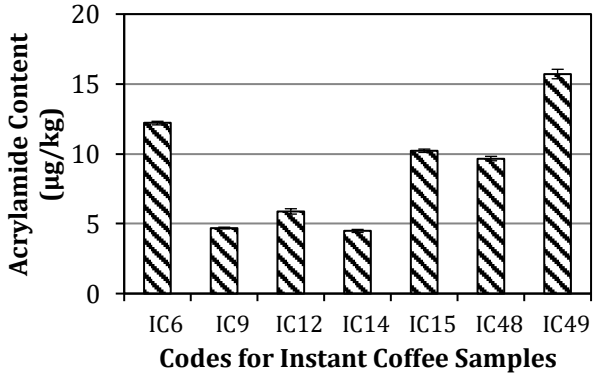

Figure 3. Acrylamide content in instant coffee samples (bars indicate standard deviations)

Table 2. Sum of analytical results for acrylamide determination in black tea, instant and Turkish coffee samples

Sample	N	Percentage of samples with acrylamide content higher than LOD (μg/kg)	Mean±SD (μg/kg)	Minimum (μg/kg)	Median (μg/kg)	Maximum (μg/kg)
Tea leaves	17	29.41	10.32±5.69	6.09	7.33	19.85
Instant coffee	27	29.63	9.01±3.89	4.48	9.43	15.71
Turkish coffee	17	41.18	30.43±32.33	3.89	23.78	88.44

LOD: Limit of detection; SD: Standard deviation

For instant coffee samples, quantification of acrylamide level was obtained in eight samples, and the range was from 4.48 to 15.71 μg/kg (Figure 2). Seven of Turkish coffee samples had a high content of acrylamide, ranging from 3.89 to 88.44 μg/kg (Figure 3).

Acrylamide contents of coffee and tea samples are mostly dependent on various factors including roasting and storage conditions. Alves et al. [25] reported that coffee species, roasting degree and brewing time had a significant influence on acrylamide contents of espresso coffee samples. Acrylamide contents of Robusta coffee were reported to be higher than Arabica coffee. They reported that prolonged roasting significantly reduced acrylamide content of coffee samples, causing the thermal degradation of acrylamide formed during roasting. Similarly, Bagdonaite et al. [26] studied the effect of roasting on acrylamide contents of different species of coffee beans and reported that acrylamide in coffee beans are formed "during the first minutes of roasting process". They reported that acrylamide formation was significantly influenced by the roasting time and temperature, species of coffee, and amount of precursors in raw material. Moreover, Arabica coffee (374 μg/kg) was reported to contain significantly lesser amounts of acrylamide than Robusta coffee (708 μg/kg). Andrzejewski et al. [27] determined the acrylamide contents of ground and instant coffee samples and reported that instant coffee samples (n=12) had an acrylamide content ranging from 169 to 539 μg/kg. LOD value was 10 μg/kg in these samples. In a study by Ölmez et al. [28], acrylamide contents of various foods sold in Turkish market were determined by the GC-MS method with a bromine derivatization step, and potato crisps had the highest acrylamide content (834 μg/kg). They also reported that Turkish coffee samples (n=4) had an acrylamide content ranging from 200 to 336 μg/kg while this range was from 95 to 402 μg/kg for instant coffee samples (n=6). Şenyuva and Gökmen [2] reported the acrylamide levels ranging from 29 to 75 μg/kg in Turkish coffees (n=5) and 42 to 338 μg/kg instant coffees (n=3). In this present study, we determined acrylamide contents in 27 instant coffee and 17 Turkish coffee samples and found that acrylamide level ranged from 4.48 to 15.71 μg/kg in eight instant coffee samples while from 3.89 to 88.44 μg/kg in seven Turkish coffee samples. The rest of the samples had a level of acrylamide smaller than the LOD value of 2.37 μg/kg. In instant coffee samples studied, acrylamide contents were usually found smaller than those reported by Ölmez et al. [28]; however, Turkish coffee samples were found similar to those reported by Şenyuva and Gökmen [2].

Reducing sugar content of coffee does not appear to influence acrylamide formation while a weak correlation was reported between acrylamide content and free asparagine concentration in green coffee beans by Lantz et al. [29]. During coffee roasting, its formation and degradation occur simultaneously, and this process may degrade most of the initially formed acrylamide. Further decay may occur mostly "through binding of acrylamide to constituents of the ground and roasted coffee matrix" during storage [30].

In instant coffee production, three steps are used in the extraction of roasted coffee beans. Since the extraction of components like carbohydrates is difficult, roasted coffee beans are passed through cells with hot water at 140-180°C under high pressure in the first step of the extraction. Then, for the extraction of aromatic compounds, coffee beans are passed at least twice from the cells which are subjected to hot water at 100°C. In the last step, the coffee extract is cooled down to about 5°C by a heat exchanger and the soluble solids content of the coffee extract is usually 20-30% [31].

Turkish coffee is generally roasted at a temperature between 204 and 218°C for a longer time than other coffee samples and roasting time is generally between 10 and 20 minutes [32]. The grain size in Turkish coffee is smaller than the others. The longer roasting time and the larger surface area of Turkish coffee than other coffee samples [32] are likely to be responsible for its higher acrylamide content than tea and instant coffee samples because the former may increase the formation of acrylamide while the latter may speed up the acrylamide transfer rate into the aqueous phase during coffee infusion preparation. Differences in grinding and brewing techniques between Turkish and instant coffee production may also influence their acrylamide contents. In addition, it has been reported that the acrylamide content in instant coffee samples may decrease during storage [26] while Turkish coffee is mostly consumed right after grinding process.

4. Conclusion

The level of acrylamide in black tea leave and instant coffee samples analyzed in this study was lower than that in the Turkish coffee samples. Approximately one third of the samples contained acrylamide at a concentration higher than the LOD value of the

method. Most of the samples had acrylamide content below the detection limit. In this present study, acrylamide analysis was carried out without any further processing of the derivatization process, and sample preparation was obtained in 2 stages (isolation + clean up). The LOD and LOQ values were similar to those reported in the literature.

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