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Monitoring of Acrylamide in Different Types of Brewed Coffee: Application of Sensitive and Efficient Analytical Technique and Method Optimization using Circumscribed Central Composite

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Abstract

Coffee is one of the favorable beverages consumed in different societies since ancient times. Variety in type and taste of this beverage take special place for coffee among families. Roasting and drying of coffee cause to form serious food contaminant named acrylamide, which have side effects on human body. Long-term exposure to this food contaminant may result in genotoxicity and carcinogencity, which necessitates analysis and monitoring its concentration in most consumed food or beverage like coffee. In this work, ingredient and heat processing have been considered as the main factors to form acry-

lamide. The concentration of acrylamide-derived in three type of coffee including ground coffee, instant coffee and coffee mix was determined by high-density solvent/microextraction joined to gas chromatography-mass spectrometry (HD-S/ME-GC-MS). The acrylamide level in coffee samples was detected in the limit of 105.09 to 1141.96 μg kg^{-1}. Acrylamide dietary exposure through coffee intake is estimated quantitatively by Monte Carlo simulation applying Crystal ball. Risk assessment results indicate that the risk of carcinogenicity of acrylamide intake through coffee consumption is considerable high in consumers and the risk of non-carcinogenicity is negligible.

Keywords: Brewed coffee; Acrylamide; Risk assessment; Analytical technique; central composite design

Graphical Abstract

Introduction

Coffee is one of the most consumed and deep-rooted brewed beverages containing unique aroma and taste in the world. Coffee has old history of usage, which has many health attributes and even bad effect on human body [1]. Caffeine and polyphenol in coffee can influence on human health because of anti-mutagenic and antioxidant activities [2]. It is reported that consumption of proper amount of coffee (3-4 cups of coffee/day) may have health effects on vigilance, alertness, cognitive powers, reducing the risk of age-related diseases such as Alzheimer and Parkinson, cardiovascular diseases, cancers, diabetes (type 2), hypertension, depression, obesity and liver diseases [1].

Arabica Coffee with sweeter and softer taste and Robusta canephora coffee with harsher and bitter taste are two species of coffee bean which accounts for 64% and 35% of the globe's coffee production, respectively [3]. Both types of aforementioned coffee have been produced in different countries such as Ethiopia, Guinea, Uganda, Brazil, Vietnam, Colombia and Indonesia while the US and Europe countries are the greatest consumer of them [1]. After harvesting the pink ripe coffee beans and removing the external layer (pericarp), several processes like drying, roasting, grinding and brewing are fulfilled to produce roasted coffee [4]. Green coffee are normally roasted at high temperatures varying between 220-250°C in different times to obtain different types of beans based on color including light, medium and dark roast with different tastes [5]. Generally, the standard time to make green coffee is 7.5 min. Obviously, the time, temperature and rate of roasting process are tree critical factors that have directly affected the organoleptic characteristics especially color, taste and aroma during maillard and caramelization reactions [6].

Roasted coffee has been classified as main source of acrylamide. Different amino acids like asparagine and ala-

nine and different sugars are the major ingredients for acrylamide production in coffee through maillard reaction based on decarboxylation and deamination depend on their varieties and rate of ripeness [7]. Also, other reactions such as amino dehydroxylation of acrylic acid at high temperature giva rise to acrylamide formation [8]. Acrylamide is mutagenic, neurotoxic, genotoxic chemical and has high healthy risk for consumers [9]. It is introduced as probable carcinogenic food contaminant for human by the International Agency for Research on Cancer (IARC) in 1994 [10]. Acrylamide can rapidly diffuse to different tissues and move throughout the whole body by blood stream. In the liver, acrylamide convert to its main active epoxide metabolite called glycideamide by monooxygenase enzymes (cytochrome P450). Acrylamide and Gycideamide, could form adduct with biomolecules in the body like DNA and hemoglobin and cause noxious effect and cancer [11, 12].

The European Committee passed a regulation establishing mitigation strategies and benchmark doses to decrease the acrylamide in foodstuffs. Based on this guidance, the levels of acrylamide in coffee samples have to be lower than 400 μg kg−1 [13]. European Food Safety Assurance (EFSA) has reported that ground coffee and instant coffee have high level of acrylamide in the range of 197–256 mg kg-1 and 229–1123 mg kg-1, respectively [14]. High level of acrylamide in coffee samples and its consumption worldwide cause a serious focus on risk assessment of this compound in societies [15].

Risk has been described as a function of hazard and exposure. Risk identification of toxic compounds has been increased in the recent years. Requirements to evaluate the risks of chemical compounds especially in food samples have been included in various regulatory frameworks in the EU, the US, as well as other regions. This widespread attention has led to the finding of emerging aspects and methodologies to evaluate exposure and detrimental effects of toxic compounds in the most consumed food products. Acrylamide risk assessment in carbohydrate-rich foods has gaining its importance in recent years. Monte Carlo methods is a precise computational algorithms. This method plans repeated random sampling to obtain numerical results. Using of this methodology to assess risk of acrylamide in food sample can be present the obvious picture of level cariogenic risk level of this toxic compound for consumer.

Extraction and determination of the exact level of acrylamide in coffee need to establish sensitive, selective and effectual sample preparation and analytical method. Because of different complexity and interferences of coffee sample, the couple of developed microextraction and chromatographic techniques can impressively extract and detect the real amount of acrylamide [11, 16]. In the last two decades, microextraction methods that used micro volume of organic solvent or green organic solvent have been applied to extract different toxic compounds from food samples. Dispersive-microextraction introduced by Rezaee and et al., is rapid, sensitive, selective and easy procedure for the extraction of acrylamide from coffee samples [17]. Complete distribution of extracting solvent throughout the sample solution cause to make the highest extraction recovery. Also, in this method, microliter volumes of extraction solvent have been used for extraction process and sensitive and precious results have been obtained.

In the present research, ingredient of three types of coffee samples and heat processing have been investigated as main factors. Exposure assessment and risk estimation through acrylamide intake via coffee consumption is performed using Monte Carlo Simulation. The acrylamide concentration in various kinds of coffee samples have been measured using high-density solvent/microextraction joined to gas chromatography-mass spectrometry (HDS/ME-GC-MS). Main factors in extracting operation have been optimized by response surface methodology (RSM) based on central composite design (CCD).

Material and Methods

Chemical solvents and Reagents

Acrylamide (> 99%) and acetamide (99%) were ordered from Merck (Darmstadt, Germany). Carbon tetrachloride, chloroform, di-potassium hydrogen phosphate, ethanol, hydrochloric acid, hydroxide potassium, methanol, potassium hexaferrocyanide, tetrachloroethylene, xanthydrol and zinc acetate were purchased from Merck (Darmstadt, Germany). Acetone, acetonitrile, sodium chloride and sodium hydroxide were obtained from Daejung Company (Shiheung, South Korea).

0.05 g of xanthydrol was dissolved in 1 mL of methanol to prepare the derivatization reagent. Carrez 1 is provided by dissolving 11 g of potassium hexaferrocyanide in 100 mL distilled water, and 22 g of zinc acetate was solved in 97 mL distilled water and 3 mL acetic acid to make carrez 2. Acrylamide standard powder was dissolved in methanol to prepare standard solution at concentration of 2000 μg mL^{-1}. Stock standards of acrylamide in the range 5-500 μg kg^{-1} were prepared. 0.1 g of acetamide was dissolved in 50 mL of methanol to prepare internal standard solution. All obtained standard solutions were placed in 4°C to apply for experiments.

Samples Collection

Fifteen types of three groups of coffee samples including 5 samples of ground coffee (3 samples: Arabica and 2 samples: Rubusta), 5 samples of instant coffee (70/30; Arabica/Rubusta, roasting level: 230 °C) and 5 samples of coffee mix (Coffee: 20%, suger: 30%, creamer: 49%, additive: 1%) were purchased from some local market (Tehran, Iran, 2024).

Acrylamide Exposure by Coffee Consumption

To evaluate whether acrylamide exposure through coffee consumption is a risk or not, exposure assessment is performed. Occurrence data including acrylamide level in coffee samples gained by ME-GC-MS as μg kg^{-1}, and consumption data containing the intake rate of coffee by Tehran population from 'The Tehran Lipid and Glucose Study' as g/day is required. Afterwards, the acrylamide exposure through coffee consumption is computed by multiplying these data and then divided by the average body weight of the Tehran population and represented as μg of acrylamide/kg BW/day [17]. Then, acrylamide dietary exposure through coffee intake is estimated quantitatively by Monte Carlo simulation applying Crystal ball version 7.2. Oracle 7291 people (3-96 years).

Risk of Acrylamide Resulted from Coffee Consumption

For investigating the risk resulted from acrylamide exposure via coffee consumption in Tehran population different approaches have been used for estimation of carcinogenicity and non-carcinogenicity risk including margin of exposure (MOE), the incremental lifetime cancer risk (ILCR) and target hazard quotient (THQ) (all of them are dimensionless) [18]. All approaches are calculated using Monte Carlo Simulation.

Extraction Method and Instrumentation

0.2 g of coffee was weighed and 0.5 mL of water was added and this sample was thoroughly mixed. Then, 200 μL of acrylamide and 200 μL of acetamide solutions were poured to sample. 7 mL of KOH (1 moL mL^{-1})-ethanol with ration 20:80 was added and shaked during 2 min. The prepared solution was centrifuged at 2683.2 g within 5 min. After separation of the clear phase, pH was modified to 3 with HCl. To remove proteins, polysaccharides and other interferences, 1 mL of each carrez solution including potassium hexaferrocyanide and zinc acetate were added into sample. After mixing and centrifuging, the clear phase was separated and 2 mL HCl (1 mol L^{-1}) and 60 μL of xanthydrol (derivatization reagent) were added. After shaking, the obtained solution was kept in dark room at room temperature for 30 min to finish the derivatization reaction. In next step, 2 mL K_2HPO_4 (2 mol L^{-1}) and 2 mL of KOH (2 moL L^{-1}) were added. Then, the sample pH was modified to 7. Then, the solution was centrifuged at 2683.2 g for 5 min and the supernatant was isolated and was used in microextraction procedure. Finally, 80 μL of extracting solvent (tetrachloroethylene) and 300 μL disperser solvent (ethanol) were added. After mixing and centrifuging, 1 μL of the bottom phase containing extracting solvent and acrylamide was injected to the GC-MS.

An HP-5 MS analytical column containing of 5% phenyl siloxane and 95% methyl polyorgano-siloxane was used (Agilent, length, internal diameter and film thickness: 30 m, 0.25 mm and 0.25 μm, respectively). The oven temperature plan was started from 100°C and increased to 300°C after 1 min with rate 20 °C min^{-1}. The temperature was stored at 300°C for 10 min. The carrier gas was Helium (He) with a contant flow rate of 0.8 mL min^{-1}. The temperature of 290°C and 280°C were regulate for the injector and auxiliary ports, respectively in a splitless injection mode with split ratio of 1:50. A 5975C inert MSD network mass selective detector was utilized. The selected ion monitoring (SIM) mode was

applied for quantification of acrylamide and acetamide. Peaks at 207 and 234 *m/z have been allocated for acrylamide and* 180 and 239 *m/z* was identified for acetamide as internal standard. The acetamide and acrylamide retention times were 9.97 and 10.30 min, respectively.

Experimental design

In this investigation, the major variables which affect microextraction step such as volume of extracting solvent (A), volume of dispersive solvent (B), salt level (C) and pH (D) were chosen. For each variable, high and low set points were designated to create an orthogonal design. Central composite design (CCD) program was utilized for optimization process to achieve the best response. Design offers 30 treatments with five levels for four variables which have six center points. Software package Design-Expert 8.0.5 (Minneapolis, USA) was used for this purpose.

Results and Discussion

Ingredient and Heat Processing Effect on the Level of Acrylamide in Coffee Sample

Figure 1 exhibits the acrylamide amount in various kinds of coffee samples. The maximum mean value of acrylamide was observed in instant coffee (849.31 μg kg^{-1}) followed by ground coffee (505.65 μg kg^{-1}) and then coffee mix (121.32 μg kg^{-1}). EFSA reported the acrylamide level in the range of 197–256 mg kg^{-1} and 229–1123 mg kg^{-1} for ground coffee and instant coffee, respectively [19] which this has good agreement with our results. Ground (roasted) coffee and instant (soluble) coffee, unlike coffee mix, are contained 100% of pure coffee bean, without addition of any irrelevant ingredients or other materials [5]. Instant coffee is made from roasted ground coffee that is extracted with hot water. Then, the obtained extract dried by spray or freeze-drying produced coffee powder which is named instant coffee. Although, coffee-mix is a type of instant coffee which contain other ingredients mainly sugar. Both instant coffee and ground coffee have been consumed as beverages which prepared by the addition of different volume of hot water [5]. Due to the polarity of acrylamide, it is completely extracted by hot water in brewing process. Hence, all of the acrylamide existing in ground and instant coffee is totally transferred to the brew [20]. It seems that the different ingredient of coffee mix sample caused to significant gap among the detected acrylamide concentration in this type of coffee and ground or instant coffee. The important reasons for the differences of acrylamide level in coffee samples are coffee type (Arabica or Robusta), ripeness of the coffee beans, rate, time and temperature of roasting, storage time and preparation procedure [14]. In addition, the asparagine level in Robusta beans is approximately higher than Arabica, which prove the higher acrylamide production in the Robusta type [1].

As was mentioned in Introduction section that green coffee beans are normally roasted at high temperatures varying between 220-250°C in different times to obtain different types of beans based on color including light, medium and dark roast with different tastes, it can be concluded that because of similar applied temperature on coffee beans, this factor is important parameter on the formation of acrylamide in coffee but the ingredient of coffee has a critical role.

Kinetic of acrylamide formation within coffee beans roasting is like reverse-U. The acrylamide level within roasting increased and then reduced sharply at the end of the roasting process due to chemical and physical decomposition. Accordingly, this occurrence may have negative impact on coffee aroma and taste [5]. It is announced that light roasted coffee beans may include higher amounts of acrylamide compared with dark roasted coffees [20].

There is no allowable value for acrylamide level in foodstuffs. The most important foods containing acrylamide are reported potato products, cereal products and roasted coffee [21]. Allowed limits noted for acrylamide concentration in drinking water is established 0.5 and 1 μg L^{-1} by World Health Organization (WHO) and European Union (EU), respectively [22]. Even though, European Committee (EC) has established some indicative values (not safety limits) for different food products which is 450 and 900 μg kg^{-1} for ground coffee and instant coffee. It is worthwhile to note that if acrylamide concentration in coffee were higher than these values, the suitable measurements should be taken to reduce acrylamide in food products especially by modifications in the roasting process [14]. The consequences of this survey depict that the acrylamide amount in

instant coffee are lower than the reported indicative value, while its content in ground coffee are higher than this amount.

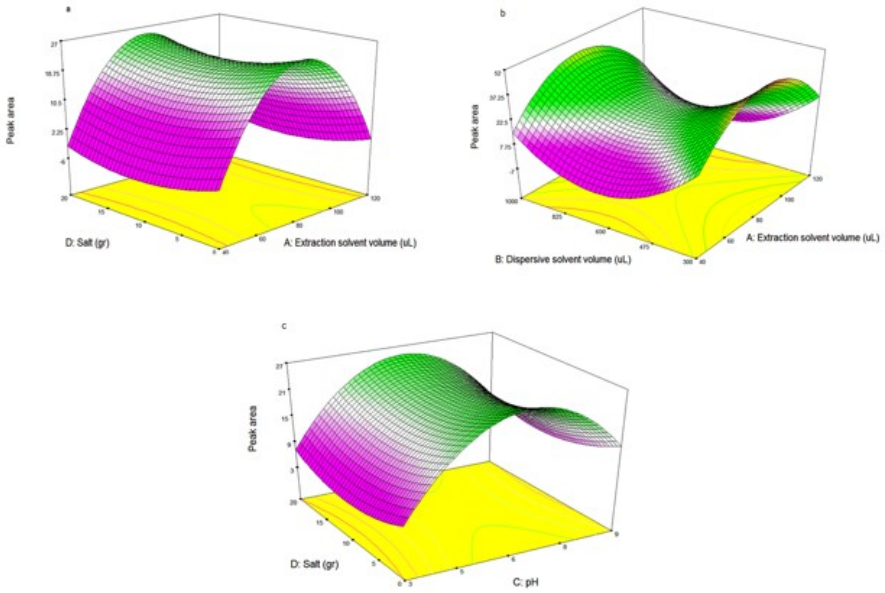

Figure 1: Acrylamide concentration (a) and mean concentration of acrylamide (b) in different types of brewed coffee.

Table 1: Results of acrylamide level in the three types of brewed coffee samples

Food Sample	Concentration (µg kg-1)	Added Amount (µg kg-1)	Analyzed Amount (µg kg-1)
Instant coffee 1	1129±45.16	100	1167.55±46.70
Instant coffee 2	1141.96±45.68	100	1179.86±47.19
Instant coffee 3	610±24.40	100	674.50±26.98
Instant coffee 4	660.10±26.40	100	722.09±28.88
Instant coffee 5	705.50±28.22	100	765.22±30.60
Ground coffee 1	697.93±27.92	100	758.03±30.32
Ground coffee 2	589.36±23.57	100	654.89±26.19
Ground coffee 3	428.26±17.13	100	501.84±20.07
Ground coffee 4	564.25±22.57	100	631.03±25.24
Ground coffee 5	248.4±9.94	100	330.98±13.23
Coffee mix 1	119.60±4.78	100	208.62±8.34
Coffee mix 2	134.90±5.40	100	223.15±8.92
Coffee mix 3	125±5.00	100	213.75±8.55
Coffee mix 4	121.20±4.85	100	210.14±8.40
Coffee mix 5	105.90±4.24	100	195.60±7.82
Mean Value ± Standard Deviation (n=3)			

Table 1 shows the concentration and analyzed amount of acrylamide in 15 various kinds of coffee which are found in the range of 105.90-1141.96 μg kg^{-1}. Standard addition method has been applied to confirm the results.

Risk Assessment

Exposure Estimation

The average acrylamide exposure for coffee in the Tehran is 0.01 μg kg^{-1} BW/day owing to low intake of coffee by individuals (1.51 g/day). P95 and P97.5 (high consumers) for this population estimated 0.04 and 0.07 μg kg^{-1} BW/day, respectively. It is observed that coffee consider as one of the main contributors in acrylamide dietary exposure in some countries due to higher consumption rate [1]. For example, in Sweden, Norway and The Netherlands, coffee has supplied 39%, 28% and 13% of the total acrylamide dietary intake, respectively [23]. When it comes to the acrylamide exposure resulted from coffee consumption, the reports observed the mean range of 0.003-0.171 μg/kg body weight (BW)/day which supplied 0.5%-41.4% of the total dietary acrylamide exposure, hanging on the country traditions [16]. However, the findings of current investigation reveal that coffee is not a main contributor of acrylamide exposure due to relatively lower consumption rate compared to European countries. Thus, in spite of high amount of acrylamide in coffee samples (especially in instant coffee), estimated acrylamide exposure is low because of low consumption rate.

Table 2: Estimated risk of carcinogenicity and non-carcinogenicity for coffee consumption.

Risk of Carcinogenicity				
	Mean	P95	P97.5	Risk Zone
MOE (170)	17000	4250	2428	Lower than 10,000
MOE (180)	18000	4500	2571	Lower than 10,000
MOE (310)	31000	7750	4429	Lower than 10,000
ILCR	8.51×10-6	3.23×10-5	5.05×10-5	Higher than 10-6
Risk of Non-carcinogenicity				
	Mean	P95	P97.5	Risk Zone
MOE (430)	43000	10750	6143	Lower than 125
THQ	0.005	0.02	0.035	Higher than 1

Risk Estimation

Table 2 shows MOE, ILCR and THQ for identification the risk of carcinogenicity and non-carcinogenicity in Tehran population for acrylamide exposure through coffee consumption as well as related safety limits. As it is obvious from this table the risk of carcinogenicity regarding to calculated MOE (170, 180 and 310) is low in the mean population whereas in high percentiles (P95 and 97.5) shows considerable risk. It is reported that MOE 10,000 or lower is indicative of high concern for population, in terms of carcinogenicity [24]. When it comes to ILCR, the results show great risk of carcinogenicity (higher than 10^{-6}) for acrylamide exposure via coffee consumption. For instance, in mean Tehran population from 1 million people, about 8 individuals probably will give cancer due to acrylamide intake via coffee during their 70 years' lifetime. However, the calculated MOE (430) and THQ represent that the risk of non-carcinogenicity (neurotoxicity) is negligible.

Micro-Analytical Method

In an effort to isolate and determine acrylamide in coffee samples, critical variables which significantly affected the extraction output in the microextraction procedure, involving kind and amount of disperser and extracting solvent, salt level and pH were optimized applying response surface methodology based on central composite design. Selection of type of disperser and extraction solvents was the initial step in the optimization. Some organic solvent such

as chloroform, carbon tetrachloride and tetrachloroethylene have been tested. The observations disclosed that tetrachloroethylene have the best extraction recoveries and acceptable chromatographic behavior compared to other solvents due to higher density and lower solvability in water. Therefore, tetrachloroethylene was chosen as extraction solvent. Good miscibility in both water and the extracting solvent is the major character for disperser solvent. Methanol, acetonitrile, acetone and ethanol were selected and studied to achieve the best solvent. Among selected disperser solvents, ethanol was showed the maximum recovery and extraction output in the microextraction. The proper miscibility of ethanol with extraction solvent and formation of ideal cloudy state in the throughput of solution causes to make high surface area interaction between extraction solvent and sample solution.

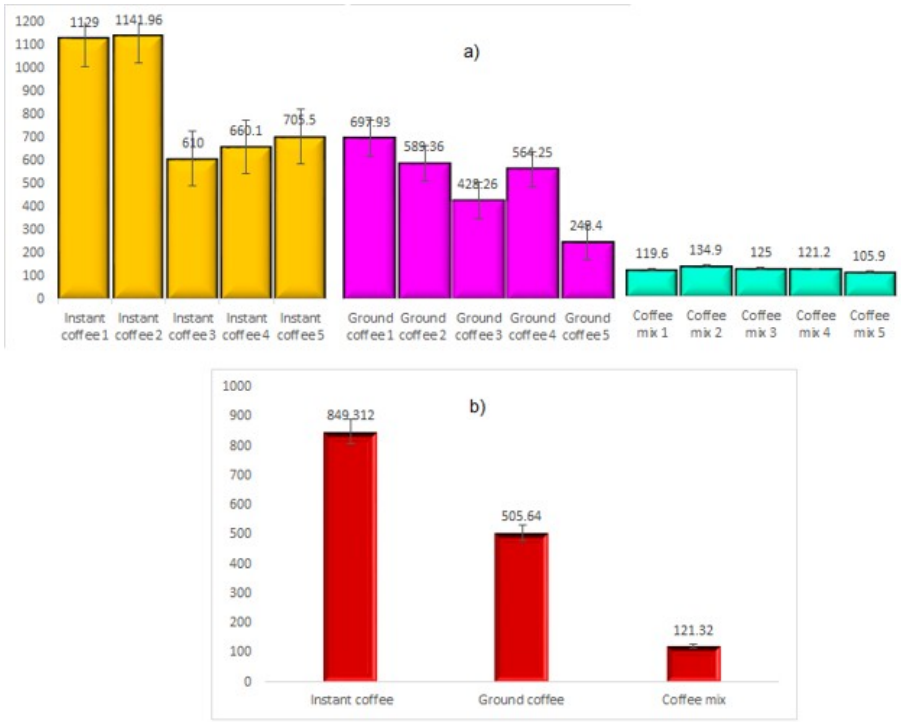

Figure 2: Response using the central composite design obtained by 3D: a) salt vs. extraction solvent volume, b) disperser solvent volume vs. extraction solvent volume, c) salt vs. pH.

Optimizing the contributing factors in the microextraction method was the next stage in the present experiment accomplished by CCD. A quadratic polynomial model was acheived to disclose a critical point including, highest, lowest or middle point and predict the respond of dependent factor for the acrylamide extraction. To achieve the experiments value, the expression k^2 +2k + C was used, where k is the factors numbers and C is the total number of central points. This design comprise of central points (C), star points (2k) and (k^2) factorial design that 30 experiments was carried out with f = 4 and C = 6 value.

The correlation coefficient value (R^2) and the adjusted correlation coefficient (adjusted-R^2) attained was 0.9474 and 0.8983, respectively which are adequately high. The observations demonstrated good accordance with experimental data and adequacy of the model. The residuals normality indicated that the errors distribution is normal. Actual and predicted responds for the acrylamide production, also demonstrated an acceptable correlation between actual and predicted responds and good accordance of the recommended quadratic model. The ANOVA also showed the significance of model (p-value 0.0001 and F-value=19.30). The lack of fit (0.73) was not considerable.

The purpose of the optimization was to achieve the optimal level of each selected variable to obtain the highest responds. For the achievement of the suitable level for each variable and assess the interaction effect of two variables on respond, three-dimensional graphs extracted from

the model were employed. Figure 2 illustrates the 3D graphs and contour plots of the major variable impress on respond. Figure 2a corroborates the positive interaction between the extraction solvent volume (80 µL) and salt level (0 g NaCl). Figure 2b indicates a remarkable interaction between the positive linear effect of the volume of dispersive and extracting solvents. The highest extraction output was attained at 80 µL of tetrachloroethylene and 300 µL of ethanol. With the augmentation of the volume of tetrachloroethylene and ethanol, because of dilution effect, the final upper phase was enhanced and the concentration of the target analyte in the upper phase was diminished. The interaction for NaCl level and pH was considerable (Figure 2c). Concurrent changes in both factors including reducing the salt to 0 g and enhancing pH to 7 gave rise to increase extraction recovery. The sample solution pH has a critical role in microextraction procedures. Therefore, the effect of pH on the extraction output of acrylamide was investigated in the limit of 3.0-9.0. The consequences showed that the highest respond was gained at pH 7 (Figure 2c). Accordingly, pH 7 is the optimum level and was chosen for the microextraction. Based on the the abovementioned findings, the optimized values were achieved: 80 µL tetra-chloroethylene; 300 µL ethanol; salt amount, 0 g NaCl and pH: 7.

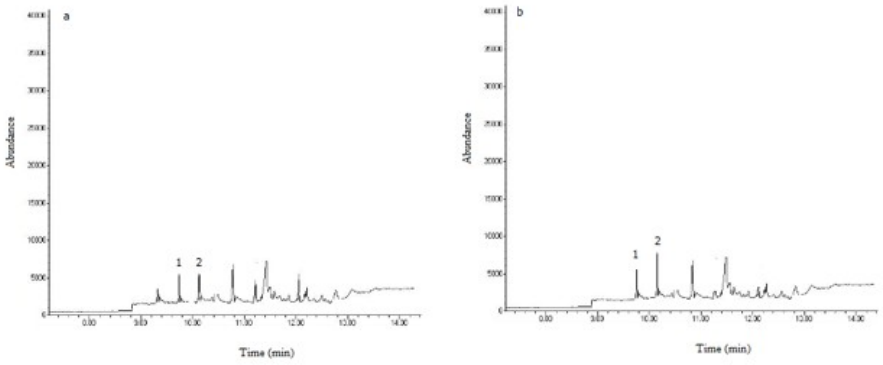

Figure 3: The chromatogram obtained by offered technique for brewed coffee sample (Coffee mix 5). (a) Non spiked and (b) spiked sample at the concentration 100 ng g^{-1} of acrylamide. 1) acetamide (internal standard) and 2) acrylamide.

Table 3: Comparison between proposed method and other methods for the determination of acrylamide in coffee powders.

Method	Sample	DLR (µg kg-1)	R2	RSD (%)	LOD (µg kg-1)	LOQ (µg kg-1)	Recovery (%)	EF	Acrylamide Level (µg kg-1)	Ref.
SPE-HPLC-FLD1	Coffee	50-1000	-	3.2	17	30	45	-	374-708	[25]
LE-SPE-GC-MS2	Coffee	0-300	0.997	46059	-	10	97.4-108.4	-	23.8-305	[29]
MSPD3/GC-MS	Coffee	0-1500	0.999	46063	5	15	84-97	-	23.6-330	[28]
SPME4-GC-MS	Coffee	0.5-200	0.999	2.2-9	0.1	0.5	-	-	502-586	[27]
SPE-LC-MS/MS5	Coffee	50-1500	0.998	8.4	-	50	97.4	-	111-114	[30]
SPE-HPLC–MS/MS	Coffee	2-100	0.9997	5	5	16	92-95	-	150-327	[26]
HDS/ME-GC-MS6	Coffee	5-500	0.998	4	0.6	2	95	116	105.90-1141.96	This study

[1]Solid phase extraction-high performance liquid chromatograph-fluorescence detector. [2]Liquid extraction- solid phase extrac-

tion-gas chromatography-mass spectrometry. [3]Matrix solid phase dispersion. [4]Solid phase microextracton. [5]Solid phase extraction- liquid chromatograph-tandem mass spectrometry. [6]Microextraction-gas chromatography-mass spectrometry.

Validation Step of Offered Method

The validation of offered technique was done by calculating linearity, repeatability (RSD %), recovery, enrichment factor (EF), limit of detection (LOD) and limit of quantification (LOQ) under optimum circumstances. The calibration curve linearity was gained 5-500 μg kg^{-1}. The correlation coefficient (R^2) was announced higher than 0.998. Furthermore, the limit of detection (LOD) and the limit of quantification (LOQ) were 0.6 μg kg^{-1} and 2 μg kg^{-1}, respectively. In an attempt to compute the repeatability, comparative peak areas calculated from six replicates experiments under optimal condition. The enrichment factor is expressed as the ratio between the analyte amount in the organic phase and the initial concentration in the aqueous solution. The enrichment factor value of the suggested method was reported 116. The recovery evaluation was accomplished by spiking 50 μg kg^{-1} of acrylamide into the various coffee samples. The acrylamide recovery was specified by comparing the amount of analyte added to the samples with the concentration after the extraction procedure. The acrylamide recovery in the current extraction method was gained as 95%. This method was authenticated by repeating the six same analysis of one sample coffee, and the RSD was calculated 4%. These consequences confirmed the adequacy of the offered method for the extraction of acrylamide from the coffee under optimal condition with high accuracy and precision. The comparison between the achievements of this research and other related studies were represented in Table 3 [25-30]. Standard addition verification was applied and acceptable isolation of acrylamide and acetamide has been achieved (Fig. 3). The chromatograms present the coffee mix sample (number 5) before (a) and after (b) spiking with the acrylamide and acetamide standards at the level of 100 ng g^{-1}.

Conclusion

Complexity of coffee matrices and different impurities cause critical problem and interest aspect for the investigation of acrylamide in coffee samples as high-consumed beverage. In the present work, an efficient and sensitive microextraction method followed by GC-MS technique have been applied and validated to measure exact level of acrylamide in various kinds of coffee. Ingredient of coffee samples showed the critical role in the production of acrylamide in coffee. According to the achieved findings, the acrylamide concentration in instant coffees is more than ground coffees and coffee mix. Acrylamide dietary exposure through coffee intake is estimated quantitatively by Monte Carlo simulation using Crystal ball. Risk assessment results represent that the risk of carcinogenicity of acrylamide intake through coffee consumption is considerable in the most of the consumers and the risk of non-carcinogenicity is negligible.

Author Contribution Statement

All of the authors contributed significantly to the research. Marzieh Kamankesh contributed to the investigation, methodology, writing - original draft, editing. Fatemeh Barzegar contributed to the manuscript editing. Amene Nematollahi contributed to the formal analysis, writing. Kiandokht Ghanati contributed to the supervision, writing - review & editing. Abdorreza Mohammadi contributed to the supervision, planning, investigation, writing-review & editing, validation.

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Conflict of Interest

The authors declare no conflict of interest.

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