INTERNATIONAL JOURNAL OF SCIENCE, TECHNOLOGY AND DESIGN

ULUSLARARASI
BİLİM, TEKNOLOJİ VE TASARIM DERGİSİ

Kahve Örneklerinde Akrilamid ve Kafein Tayini İçin Alternatif Metot Çalışması

Büşra KURUCA[1], Belgin İZGİ[2]

[1] *B.U.Ü., Faculty of Arts and Science, Chemistry Department, Undergraduate Student, Bursa, TURKEY, ORCID ID 0000-0002-9177-6992*
[2] *B.U.Ü., Faculty of Arts and Science, Chemistry Department, Bursa, TURKEY, ORCID ID 0000-0002-1074-3612*

Corresponding Author: Büşra KURUCA, kuruca.5263@gmail.com

Özet

Akrilamid (AA), son yıllarda dikkatleri üzerine toplayan, gıdaların ısıl işlem süreci sonucunda ortaya çıkan bir bileşimdir. 1994 yılında Uluslararası Kanser Araştırmaları Ajansı (IARC) tarafından Grup 2A'da 'insan için muhtemel karsinojenik madde' olarak listelenmiştir. Kafein (CA), önemli fizyolojik etkileri olan alkaloid grubunun azotlu bir organik bileşiğidir. Akrilamid ve kafein tayini için literatürde birkaç analitik yöntem vardır. Bu yöntemler GC-MS, LC-MS, voltametri, iyon kromatografisi, FT-IR, GC-EDC, LC-MS/MS ve GC-IT/MS'dir. Ek olarak, organik moleküller metallerle kompleksleşme yoluyla tanımlanabilir. Job Metodunun belirli bir karışımda bulunan metaller ve organik moleküller arasındaki kompleksin oluşum hızını belirlediği iyi bilinmektedir. Bu projede, kahve örneklerindeki CA ve AA miktarını belirlemek için alternatif bir yöntem denendi. Öncelikle, CA ve AA'nın Cu ve Co ile oluşturduğu metal kompleksleri UV-VIS Spektrofotometresi ile ölçüldü ve M:L oranı Job Metoduna göre 1:1 olarak belirlendi. Optimum koşullar ayarlandıktan sonra, standart çözeltiler ve kahve örnekleri 252.0 nm'de (CA için) ve 272.0 nm'de (AA için) ölçüldü. Elde edilen verilere dayanarak, ticari kahve örneklerinde akrilamid ve kafein miktarı belirlendi.

Article Info

Research Article
Received: 20/07/2020
Accepted: 21/08/2020

Anahtar Kelimeler

Akrilamid, kafein, Job yöntemi, spektrofotometri, kahve

Öne Çıkanlar

UV-VIS Spekrofotometresi, Job Yöntemi, Spektrofotometri.

Development of alternative methods for determination of acrylamide and caffeine in coffee samples

Abstract

Acrylamide which has gained attention in recent years is a composition resulting from the heat treatment process of foods. By the IARC, it is listed in Group 2A as a possible carcinogenic substance for humans. Caffeine is a nitrogenous natural compound of the alkaloid group, which has crucial physiological effects. There are numerous analytical methods within the literature for acrylamide and caffeine determination. These methods are GC-MS, LC-MS, voltammetry, ion chromatography, FTIR, GC-EDC, LC-MS/MS, and GC-IT/MS. Also, organic molecules could be identified via the complexation with metals. It is well known that the Job Method is determining the formation rate of the complex between metals and organic molecules in a certain mixture. In this project, an alternative method was tried to determine the CA and AA amount in coffee samples. First of all, the metal complexes of CA and AA formed with Cu and Co were determined as 1:1 according to Job's Method by UV-VIS Spectrophotometer. After optimum conditions were set, standard solutions and coffee samples were measured at 252.0 nm (for CA) and 272.0 nm (for AA). Based on the data obtained, the amounts of acrylamide and caffeine were determined in commercial coffee samples.

Keywords

Acrylamide, caffeine, Job's method, spectrophotometry, coffee

Highlights

UV-VIS Spectrophotometer, Job Methods, Spectrophotometry

1. Introduction

1.1. About acrylamide

Acrylamide is a white crystalline solid under room temperature. Acrylamide, which includes a reactive electrophilic double bond and amide group, can show off both weakly acidic and basic properties (Yıldız 2014). It is a compound that gained extensive attention in recent years because it was discovered to be formed as a result of warmth processing in foods. Acrylamide has chemical components of C_3H_5NO, and its IUPAC name is 2-propenamide (Ölmez et al 2008; Alpözen et al 2012).

Acrylamide is available in two forms, monomer and polymer. Acrylamide, which is in a monomer form, is toxic to the nervous system and is thought to be carcinogenic to humans. The toxic effect of acrylamide in polymer forms is unknown (Arusoğlu 2015; Yıldız 2014).

Acrylamide is electrophilic because it has an unsaturated carbonyl group in its structure and therefore it can easily react with nucleophilic groups commonly found in biological molecules such as carboxylates, amines and thiols (Yıldız 2014).

Acrylamide became classified as " probably carcinogenic to humans" (Group 2A) by the International Agency for Research on Cancer (IARC, 1994) and it becomes detected in carbohydrate-rich fried or baked meals samples by the research group from the Swedish National Food Administration (SNFA) and the University of Stockholm in 2002 (Hellenäs et al 2013). Acrylamide cause of permanent genetic damage in both humans and animals. Long-term studies have been conducted to see the effect of acrylamide on rats and mice. This study showed that the frequency of tumors in varied organs increases when the animals are exposed to acrylamide. According to these studies, attempts have been tried to be determined the possibility of developing that a human will contract cancer due to acrylamide exposure (Hellenäs et al 2013).

Many studies have been done to understand the mechanism of formation of this chemical after acrylamide has been included in the "probable carcinogen for humans" substance group by IARC (Eriksson 2005). Although not all types of creation of acrylamide are known, it is known to be formed mainly by the Maillard reaction and by some minor ways other than the Maillard reaction, although more limited (Akgün 2019). In addition, acrylamide can be formed from the acrolein compound, nitrogenous compounds, 3-amino propionamide and oxidized lipids (Gökmen et al 2006; Doğan et al 2006).

There are many studies in the literature to determine the acrylamide level of foods. According to these studies, 90% of dietary acrylamide intake results from products such as french fries, potato chips, coffee, bread, biscuits and breakfast cereals (Svensson et al 2003; Konings et al 2003). Coffee beans are exposed to higher temperatures than other foods. Therefore, other ways along with the Maillard reaction in coffee cause acrylamide formation too (Akgün 2019).

The main substances involved in the formation of acrylamide in coffee are thought to be sucrose and asparagine. The amount of asparagine of Robusta coffees is higher than that of Arabica coffees, and the amount of acrylamide is generally higher in Robusta coffees. The average amount of acrylamide in medium roasted Arabica coffees was found to be 230 µg / kg, and in Robusta coffees was around 500 µg / kg ‘‘Alves et al 2010’’. Roasting time and speed affect the physical properties of coffee such as taste and color and the amount of acrylamide (Akgün 2019).

There are several analytical methods for acrylamide in literature. Liquid chromatography tandem mass spectrometry (LS-MS/MS), headspace solid-phase micro extraction (HS-SPME) followed by gas chromatography-flame ionization detection (GC-FID), gas chromatography coupled with electron capture detector (GC-ECD) and ion trap mass spectrometry detector (GC-IT/MS), dispersive liquid-liquid micro extraction (DLLME) followed by GC-ECD, high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) methods have been reported so far for acrylamide analysis (Altunay et al 2016).

1.2. About caffeine

Caffeine is an alkaloid of the methyl xanthine family. In its pure form, it is a bitter white powder. It is found in the structure of many plant species worldwide (leaves, seeds, fruits, etc.). The most common sources of caffeine are coffee, cocoa beans and tea leaves. It is a naturally occurring substance. Its chemical formula is $C_8H_{10}N_4O_2$, its systematic name is 1,3,5-trimethylxanthine (Wanyika et al 2010).

The effect of caffeine on the central nervous system is quick. It has also been observed to increase heart rate, widen blood vessels, and increase free fatty acid levels in plasma. One gram of caffeine is known to cause insomnia, irritability, nausea, tinnitus, and tremors (Bhawani et al 2015).

Caffeine also raises blood sugar levels as a result of accelerating breathing. It causes the blood vessels of the brain to constrict, thereby reducing blood flow to the brain. Caffeine can cause benign breast diseases (non-cancerous). It can worsen premenstrual symptoms in women who consume caffeine too much (Wanyika et al 2010).

Caffeine also stimulates the brain with similar mechanisms by increasing the release of dopamine, the hormone of happiness, such as amphetamines, cocaine and heroin. The reason why caffeine is addicted to humans depends on this. (Kitiş, 2011).

Caffeine is found in some of the foods and beverages we consume in daily life. Coffee is a well-known example of caffeine sources (Belay et al 2008). The exact amount of caffeine, contained in coffee, depends on the type of coffee bean used (Robusta beans are known to have much more caffeine than Arabica beans), the coffee brewing method used, the roasting time of the coffee beans, the extraction time and the temperature of the water used. In general, roasted coffee has less caffeine amount than lighter roasts. Roasting reduces the amount of caffeine in coffee beans, so dark-roast coffee has less caffeine than lighter roasts (Espresso Coffee Guide 2008).

Another commonly used source of caffeine is tea. Tea actually contains more caffeine than coffee, but since the brewing type is weaker, a typical serving contains much less caffeine than coffee. In addition to the brewing type, the caffeine content of tea is influenced by various variables, including the growth conditions of tea and tea processing techniques. The amount of caffeine in tea varies according to the type of tea. The tea preparation stage and many other factors have an important effect on the color of the tea, a very weak indicator of caffeine content (Wanyika et al 2010).

There are several analytical methods including spectrophotometry for caffeine in literature. High-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS/MS), voltammetry, Fourier transform infrared spectrophotometry (FT-IR), chemiluminescence, gas chromatography-mass spectrometry (GC-MS), ion chromatography, capillary electrophoresis, and ultra-high-performance liquid chromatography (UPLC) methods have been reported so far for caffeine analysis (Turak et al 2017).

In addition to all these techniques, UV-VIS spectrophotometry can be used, which is known to provide high accuracy and repeatability with a small number of samples. This

method is widely used in many areas as it is cheaper and follows a simpler procedure than other techniques. Almost all researchers can use this device (Bhawani et al 2015).

1.3. Continuous variation method

The Job method, also called the continuous variation method, is used to determine the stoichiometry of a metal-ligand complex ‘‘Harvey 2013’’. Applications of Job method, problems related to organometallic chemists are explained. The Job method provides qualitative and quantitative information to stoichiometry, which is the basis of the combination of the m molecules of A and the n molecules of B to form A_mB_n (Renny et al 2013).

Let's assume that a single complex is formed between the metal ion and the ligand and only this complex absorbs light at the selected wavelength. A series of solutions are prepared in which the total of the ligand concentration C_L and the metal ion concentration C_M is kept constant ($C_T = C_L + C_M$), and the absorbance of the complex is measured for each solution (Taylak Bağdu, 2009).

These absorbance values are plotted against the mole fraction of the metal ion or ligand. Two straight segments of the curves obtained are extended by extending the rising and falling sections. The ratio of mole fractions corresponding to the cut point gives the metal ion/ligand ratio found in the formula of the XM / XL complex. If this value is 0.5, then ML, if this value is 0.33, then ML_2, if this value is 0.25, then ML_3, if this value is 0.20, then ML_4 complexes are formed (Taylak Bağdu, 2009).

2. Material and method

2.1. Material

2.1.1. Chemicals

Co (1000 mg L^{-1}) and Cu (1000 mg L^{-1}) Merck standards were used to apply the Job Method. Pure acrylamide (Merck) and pure caffeine (Merck) solids were used for stock solutions. Acrylamide (AA) and caffeine (CA) stock solutions and coffee samples were prepared with distilled water. pH adjustments were made using 0.05 mol L^{-1} and 0.1 mol L^{-1} NaOH solution, 0.1 mol L^{-1} HCl solution, and standard pH 3, pH 5, pH 7 and pH 10 buffer solutions.

2.1.2. Instruments

Thermo Scientific ORION 3 STAR pH meter was used for pH measurements to determine the optimum conditions. Spectrophotometric measurements of the standards and the coffee samples were made with 1 cm wide quartz cuvettes with VWR UV-6300 PC Double Beam Spectrophotometer.

2.2. Method

2.2.1. Preparation of standard solutions

To prepare AA and CA stock solutions (1.10^{-2} mol L^{-1}), 0.0355 g AA and 0.097 g CA were weighed, after dissolving in a beaker with small portion of deionized water, it was transferred into volumetric flasks and completed to 50 mL.

2.2.2. Experimental procedure

1 mg L^{-1} AA, 1 mg L^{-1} CA and a mixture of containing 1 mg L^{-1} AA and CA solutions were prepared. A wavelength scanning was done on UV-6300 PC Double Beam Spectrophotometer (*Table.1*). AA and CA solutions were prepared in certain mixture (0.01, 0.1, 0.5, 1, 5, 10, 20 mg L^{-1}). An absorbance's of the each solutions were measured at determined wavelengths and calibration graphs were drawn for each wavelength. According to the correlation coefficients, the optimum wavelengths for AA and CA were accepted as 272.0 nm (R^2= 0.9953) and 252.0 nm (R^2= 0.9986), respectively. The calibration graph of selected wavelengths were given in Figure.1.

Table.1 A wavelength ranges for AA, CA and AA-CA mixture

	C (mg L^{-1})	***λ_{max}***	
AA	*1*	*196.0 nm – 216.0 nm – 272.0 nm*	
CA	*1*	*204.0 nm – 252.0 nm – 272.0 nm*	
Mixture	*AA : CA*	***AA***	***CA***
		202.0 nm – 272.0 nm	*252.0 nm – 272.0 nm*

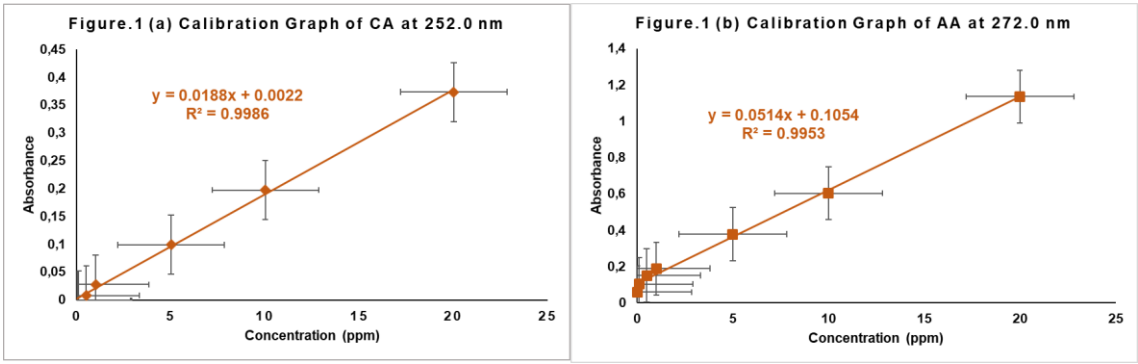

Figure.1 The calibration graphs of (a) CA and (b) AA solutions at optimum wavelengths

Job's Method (continuous variation method) was tried at different pH values to find the optimum conditions for AA-Co, AA-Cu CA-Co and CA-Cu complexes (*Table.2*). It was seen that the measurements taken were more suitable for the Job Method at pH 5.

Table.2 M:L ratios obtained at different pH values

pH	***Co***		***Cu***	
	AA	***CA***	***AA***	***CA***
3	*1:4*	*1:3*	*1:3*	*1:1*
5	*1:1*	*1:1*	*1:1*	*1:1*
7	*1:2*	*1:4*	*1:4*	*1:2*
10	*1:4*	*1:2*	*1:4*	*1:2*

Co-L and Cu-L solutions were prepared for the calibration curve at identified conditions. Absorbances of these two solutions were measured at 252.0 nm and 272.0 nm, and calibration graphs were drawn (*Figure.2*). LOD-LOQ values and the standard deviation of the least concentration were calculated (Table.3). Sample preparation was done according to respective LOD values.

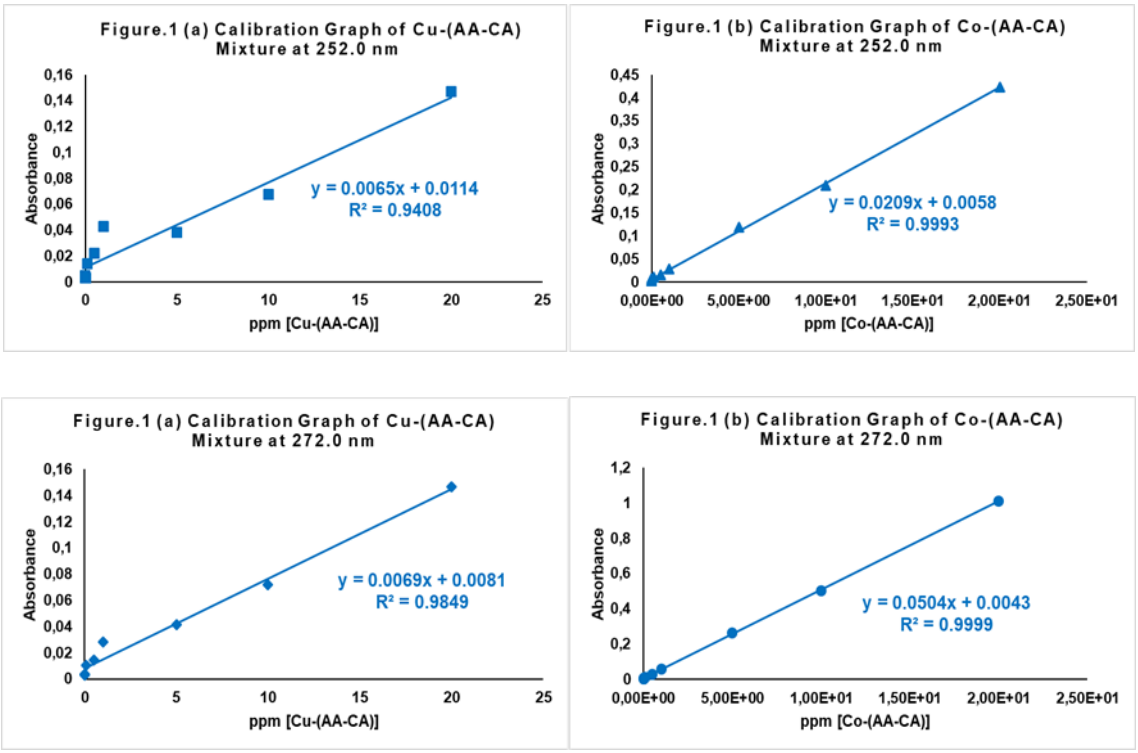

Figure.2 The calibration graphs of (a) Cu– (AA-CA) and (b) Co– (AA-CA) mixtures at identified wavelengths

Table.3 LOD-LOQ values of complex formation procedure

	Co-(AA-CA)		***Cu-(AA-CA)***	
	252 nm	***272 nm***	***252 nm***	***272 nm***
LOD	*0.0790*	*0.0290*	*0.7301*	*0.3554*
LOQ	*0.2620*	*0.0960*	*2.4338*	*1.1849*
R^2	*0.9993*	*0.9999*	*0.9410*	*0.9849*
SD of > C	*0.0005*	*0.00095*	*0.0000*	*0.0001*

2.2.3. Sample preparation

Five different commercial Turkish coffees were provided, from a low-cost brand and from four commonly consumed brands in Turkey (*Table.4*). Approximately 0.5 g of weighing was taken from each coffee sample and a known Turkish coffee cooking process was applied with 59 mL of pure water (59 mL = about 1 volume of Turkish coffee cup). All

coffee samples prepared without adding sugar. After the coffee samples had cooled, they were filtered with black tape filter paper. 1 mL of sample was added to 10 mL falcon tubes from each coffee sample. The spike concentration of metals was selected as 1 mg L^{-1} Co according to the calculated LOQ values. After providing optimum pH conditions (pH 5.0 - 6.0), the volume was completed to 10 mL. The same processes were carried out for Cu metal also. The absorbance of the samples was measured at the identified wavelengths (252.0 nm and 272.0 nm).

Table.4 Coffee samples details

Coffee Brand	***Sample Code***	***Weighing (g)***	***Volume (mL)***
1	*MK*	*0.5202 ± 0.0003*	*59 mL*
2	*HK*	*0.5144 ± 0.0001*	
3	*KK*	*0.5020 ± 0.0003*	
4	*KMK*	*0.5026 ± 0.0001*	
5 (caffeine-free)	*KMK'*	*0.5016 ± 0.0002*	

3. Results and discussion

The AA and CA values of the samples whose absorbance were measured were calculated using the equation of lines from the graphs given in Figure.2 (*Table.5*).

Table.5 Results of AA and CA contents of samples (n=3)

Sample Code	***with Co***		***with Cu***	
	CA (mg L^{-1})	***AA (mg L^{-1})***	***CA (mg L^{-1})***	***AA (mg L^{-1})***
MK	*0.35 ± 0.0015*	*0.62 ± 0.0038*	*2.07 ± 0.0128*	*2.75 ± 0.0279*
HK	*1.68 ± 0.0032*	*0.11 ± 0.0005*	*0.97 ± 0.0063*	*1.85 ± 0.0133*
KK	*0.23 ± 0.0009*	*1.39 ± 0.0002*	*2.11 ± 0.0104*	*2.12 ± 0.0154*
KMK	*0.56 ± 0.0014*	*0.29 ± 0.0001*	*2.54 ± 0.0145*	*2.21 ± 0.0152*
KMK'	*< LOQ*	*0.19 ± 0.0003*	*< LOQ*	*2.28 ± 0.0005*

Throughout the analysis, all measurements were carried out at ambient temperature (appr. 25 ° C) and performed at different pH values. The pH value most suitable for the Job

Method was accepted in the range of 5.0-6.0 (Table.2) and analyzes were performed in this optimum range.

The recovery test of all of the samples was calculated according to formula (F.1). The recovery % results for all samples were between 56.429 to 135.714 for CA and 95.556 to 111.111 for AA.

$$\text{Recovery \%} = \frac{\text{Absorbance of the spike-Absorbance of the sample}}{\text{Absorbance of the standard}} \times 100 \qquad \text{(F.1)}$$

Acrylamide and caffeine analysis can be done with different methods in coffee samples. A few examples of these methods were given below (Table.6).

Table.6 Other methods for AA and CA determination

SAMPLE	***METHOD***	***REFERENCES***
Roasted Coffee (for AA)	GC-MS	Alves et al 2010
Soluble Coffee (for CA)	ESTASI-MS	Tobolkina et al 2014
Roasted Coffee (for AA)	LC-MS/MS	Bortolomeazzi et al 2012
Coffee Beans (for CA)	FT-MIR-ATR Spectroscopy	Yisak et al 2018
In This Work	Cu and Co Complexation of AA and CA	

4. Conclusion

In this work, an alternative method was tried to determine the AA and CA amount in coffee samples. First of all, the metal complexes of acrylamide and caffeine formed with copper and cobalt were determined as 1:1 according to Job's Method. After optimum conditions were setted, standard solutions and coffee samples were measured at 252.0 nm (for CA) and 272.0 nm (for AA). Based on the data obtained, the amount of acrylamide and caffeine were determined in commercial coffee samples.

According to Food and Agriculture Organization and World Health Organization, the amount of CA and AA that can be safely consumed has been determined as for adults 400 mg L^{-1} per day (for pregnancy 200 mg day^{-1}) and 0.3 to 0.8 µg kg body $weight^{-1}$ day^{-1}, respectively (WHO 2002; FAO 2003).

When the results compared with these values, it was observed that the amount of acrylamide was higher than the allowable values. The last table show that all coffee samples results of AA depends on the heat treatment steps from the bean to the product. So that, it is clear that in the future, the parameters of analysis of the coffee's roasting time, sugar addition and coffee cooking time should be examined.

Acknowledgments
This project was realized within the scope of the KIM 4004 Graduate Practice II course. Some parts the results of this work was presented as a poster (PP19-

http://icabc2020.firat.edu.tr/documentation/Proceeding_Book2ndICABC2020.pdf) at the "2nd International Congress on Analytical and Bioanalytical Chemistry" congress on 11-14 March 2020.

Symbols
GC-MS: Gas chromatograpyh - mass spectroscopy
GC-EDC: Gas chromatography coupled with electron capture detector
GC-IT/MS: Gas chromatography - İon trap mass spectrometry
ESTASI-MS: Electrostatic spray ionization - mass spectrometry
LC-MS/MS: Liquid chromatography - tandem mass spectroscopy
LC-MS: Liquid chromatograpyh - mass spectroscopy
FT-MIR-ATR: Fourier transform mid-infrared attenuated total reflectance spectroscopy
FT-IR: Fourier transform infrared spectrophotometry
UV-VIS: Ultraviolet–visible spectrophotometry

5. References

Akgün,B. 2019. Türk kahvesinin akrilamid içeriği ile asparaginaz enziminin akrilamid oluşumu ve uçucu bileşikler profiline etkileri. Doctoral Thesis, YTU, Graduate School of Natural and Applied Science, Food Engineering Department, İstanbul.

Alpözen, E., Güven, G. 2012. Akrilamid analiz yöntemleri. *Analiz 35*. 4(13):12-16.

Altunay, N., Gürkan, R., Ulaş, O. 2016. A pre concentration method for indirect determination of acrylamide from chips, crackers and cereal-based baby foods using flame atomic absorption spectrometry. *Talanta* ,161, 143-150.

Alves, R. C., Soares, C., Casal, S., Fernandes, J. O., Oliveira, M. B. P. 2010. Acrylamide in espresso coffee: Influence of species, roast degree and brew length. *Food Chemistry*, 119(3):929-934.

Anonymous. 1994. Monographs on the evaluation of carcinogenic risk to humans: some industrial chemicals. *World Health Organization International Agency for Research on Cancer (IARC)*, vol: 60, Lyon.

Anonymous. 2002. Health Implications of Acrylamide in Food. World Health Organization, https://apps.who.int/iris/bitstream/handle/10665/42563/9241562188.pdf?sequence=1 - (07.03.2020).

Anonymous. 2008. How much caffeine in a cup of coffee?. Espresso Coffee Guide, https://espressocoffeeguide.com/how-much-caffeine-in-a-cup-of-coffee/-(25.04.2020).

Arusoğlu, G. 2015. Akrilamid oluşumu ve insan sağliğina etkisi. *Akademik Gıda*, 13 (1): 61-71.

Belay, A., Ture, K., Redi, M., Asfaw, A. 2008. Measurement of kafein in coffee beans with UV/vis spectrometer. *Food Chemistry*, 108(1): 310-315.

Bhawani, S.A., Fong, S.S., Ibrahim, M. N. M. 2015. Spectrophotometric analysis of caffeine. *International Journal of Analytical Chemistry*, 2015(6):1-7.

Bortolomeazzi, R., Munari, M., Anese, M., Verardo, G. 2012. Rapid mixed mode solid phase extraction method for the determination of acrylamide in roasted coffee by HPLC-MS/MS. *Food Chemistry*, 135(4): 2687-2693.

Doğan, İ.S., Meral, R. 2006. Gıdalarda akrilamid ve önemi. Türkiye 9. Gıda Kongresi, 24-26 Mayıs 2006, Bolu.

Eriksson, S. 2005. Acrylamide in food products: Identification, formation and analytical methodology. Doctoral Thesis, SU, Department of Environmental Chemistry, Sweden.

Gökmen, V., Şenyuva, H. Z., Dülek, B., Çetin, E. 2006. Computer vision based analysis of potato chips - A tool for rapid detection of acrylamide level. *Molecular Nutrition and Food Research*, 50(9): 805-810.

Harvey, D. 2013. Method of continuous variations. https://community.asdlib.org/imageandvideoexchangeforum/2013/07/29/method-of-continuous-variations/- (27.04.2020).

Hellenäs, K.E., Fohgelberg, P., Fäger, U., Busk, L., Zetterberg, L.A., Ionescu, C., Färnstrand, J.S. 2013. Acrylamide in Swedish food. National Food Agency, Sweden.

Kitiş, F. 2011. İlaç numunelerinde kafein ve parasetamol'ün kemometrik yöntemlerle tayinleri. Master Thesis, SDU, Graduate School of Natural and Applied Science, Chemistry Department, Isparta.

Konings, E. J. M., Baars, A. J., Van Klaveren, J. D., Spanjer, M. C., Rensen, P. M., Hiemstra, M., van Kooij, J.A., Peters, P. W. J. 2003. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Food and Chemical Toxicology*, 41(11):1569-1579.

McCabe, T. 2003. Drinks with high caffeine content. Food and Agriculture Organization of the United Nations, http://extwprlegs1.fao.org/docs/html/uk4 7802.htm - (07.03.2020).

Ölmez, H., Tuncay, F., Özcan, N., Demirel, S. 2008. A survey of acrylamide levels in food the Turkish market. *Journal of Food Composition and Analysis*, 21(7): 564–568.

Renny, J. S., Tomasevich, L. L., Tallmadge, E. H., Collum, D.B. 2013. Method of continuous variations: applications of job plots to the study of molecular associations in organometallic chemistry. *Angewandte Chemie International Edition in English*, 52(46):11998-12013.

Svensson, K., Abramsson, L., Becker, W., Glynn, A., Hellenäs, K. E., Lind, Y., Rosén, J. 2003. Dietary intake of acrylamide in Sweden. *Food and Chemical Toxicology*, 41(11):1581-1586.

Talyak Bağdu, C. 2009. Bazi geçiş metallerinin birlikte spektrofotometrik tayininde çok bileşenli kalibrasyon tekniklerinin uygulanmasi**,** Master Thesis, AÜ, Graduate School of Natural and Applied Science, Chemistry Department, Ankara

Turak, F., Güzel, R., Dinç, E. 2017. Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultra-performance liquid chromatography. *Journal of Food and Drug Analysis*, 25(2):285-292.

Tobolkinaa, E., Qiaoa, L., Rousselb, C., Girault, H.H. 2014. Standard addition strip for quantitative electrostatic spray ionization mass spectrometry analysis: Determination of caffeine in drinks. *Talanta*, 130:377-381.

Wanyika, H.N., E.G. Gatebe, L.M. Gitu, E.K. Ngumba and C.W. Maritim, 2010. Determination of Caffeine content of tea and instant coffee brands found in the Kenyan market. *African Journal of Food Science*, 4(6): 353-358.

Yıldız, A. 2014. Kati faz ektraksiyon metodu ile lc/ms-ms cihazi kullanilarak işlenmiş gidalarda akrilamid tayini ve çeşitli ön işlemlerin patates kizartmasindaki akrilamid oluşumu üzerindeki etkisi. Doctoral Thesis, DU, Graduate School of Natural and Applied Science, Chemistry Department, Diyarbakır.

Yisak, H., Redi-Abshiro, M., Chandravanshi, B.S. 2018. Selective determination of caffeine and trigonelline in aqueous extract of green coffee beans by FT-MIR-ATR spectroscopy. *Vibrational Spectroscopy*, 97:33-38