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Determination of Acrylamide Levels of Light Biscuit by LC-MS/MS

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ABSTRACT

Acrylamide, a neurotoxic compound, was classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to human. Acrylamide occurs naturally in starch based heated foods. A rapid and reliable liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method was used for the determination of acrylamide in light biscuit (cookie) samples. The method was linear up to 750 µg/kg food. Recovery rate was found as 99.66% with the limit of detection and the limit of quantification values of 1.5 µg/kg and 5.0 µg/kg, respectively. Ten samples of light biscuits supplied from markets were analyzed to determine acrylamide levels by LC-MS/MS method. Acrylamide contents of light biscuit samples varied from 42.57 µg/kg to 877.57 µg/kg with an average content of 394.47 µg/kg. This work is the first survey study for the determination of acrylamide contents of different light biscuits by LC-MS/MS method.

Key Words: Acrylamide, Light biscuit, Liquid chromatography tandem mass spectrometry, LC-MS/MS

Light Bisküvilerde LC-MS/MS ile Akrilamid Düzeylerinin Belirlenmesi

ÖZET

Nörotoksik bir bileşik olan akrilamid Uluslararası Kanser Araştırma Örgütü (IARC) tarafından insanlarda kanser yapma olasılığı bulunan bileşikler grubunda sınıflandırılmıştır. Akrilamid nişasta içeren ısıl işlem görmüş gıdalarda kendiliğinden oluşmaktadır. Bu araştırmada, light bisküvilerdeki akrilamid düzeylerinin belirlenmesinde pratik ve doğru LC-MS/MS yöntemi kullanılmıştır. Yöntemin lineerliği 750 µg/kg'dır. Yöntemin geri kazanımı %99.66, saptama sınırı (LOD) 1.48 µg/kg, ölçme sınırı (LOQ) 4.93 µg/kg'dır. Marketlerden temin edilen 10 farklı light bisküvide akrilamid düzeyleri LC-MS/MS yöntemi ile belirlenmiştir. Light bisküvi örneklerinde akrilamid düzeyleri 42.57 µg/kg ile 877.57 µg/kg arasında değişirken, ortalaması da 394.47 µg/kg'dır. Bu çalışma ile light bisküvilerde akrilamid düzeyleri ilk kez LC-MS/MS yöntemi ile belirlenmiştir.

Anahtar Kelimeler: Akrilamid, Light bisküvi, Sıvı kromotografisi tandem kütle spektrometresi, LC-MS/MS

INTRODUCTION

In April 2002, the formation of acrylamide in starch-rich foods or high-temperature cooking, like with a variety of baked and fried foods cooked at high temperature, was reported by researchers from the Swedish National Food Administration (SNFA) [6]. A number of theoretical mechanisms have been proposed for the formation of acrylamide. Maillard browning reaction was reported as the most probable mechanism for the development of acrylamide in cooked foodstuffs [4]. Asparagine is found to be the main precursor of acrylamide. Detection of high concentrations of acrylamide is common in heated starch-rich foods, which shows strong relation of

acrylamide formation to the sugar content, especially glucose and fructose [4, 9].

Acrylamide, a neurotoxic compound, was classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to human [7]. The discovery of acrylamide in human foods led to surveys exploring the levels of this potentially hazardous chemical and spurred search into suitable analytical procedures for its determination in foodstuffs. Gas chromatography with mass spectrometric detection (GC-MS) with or without derivatization, and high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) appear to be the most commonly used methods. GC-MS methods with derivatization are hazardous to health due to bromination, laborious, and time consuming. GC-MS methods without derivatization step appear to be relatively simple, but their drawbacks are the lack of characteristic ions in the mass spectrum. Nowadays, more attention is paid to LC-MS/MS techniques having high sensitivity and saving time. There are also significant differences between procedures at sample preparation step, namely extraction of acrylamide from the food matrix and clean-up of the extracts to exclude co-extractive interferences [6, 10]. When examined the previous studies on acrylamide analysis by LC-MS/MS method, it is seen that these methods include long extraction and clean-up steps and interfering peaks are observed especially when working with potato-based foods.

Biscuits are a popular food eaten by both children and adults; however, they are typically high in the materials (fat and sugar) that make them "unhealthy". In the manufacture of biscuit dough, it is traditional to use fat which is semi-solid at room temperature e.g. palm oil which contains 50% saturated fatty acids. In addition, the biscuit market is dominated by short dough biscuits having fat levels in excess of 20%. Biscuits are therefore an obvious choice when consumers are asked to reduce their total fat intake. The development of a commercially viable biscuit attractive to children and adults that will have a significant reduction in fat, with fewer calories and contain nutrients designed to reduce the risk of coronary heart disease is highly desirable [5]. As, biscuit contain sugar and protein and high heat treatment is applied during process; formation of acrylamide is expected. The aim of the present study was to determine acrylamide content of light biscuit samples which have a important place in human diet.

MATERIAL and METHODS

Materials

Samples

Ten light biscuits samples were purchased from local bakeries in Izmir (the biggest city in the Aegean region of Turkey). The samples were homogenized and stored at -18˚C until analysis.

Reagents

Acrylamide was obtained from Sigma (Steinhein, Germany). 2,3,3-D_3-labelled acrylamide (D_3-AA, isotopic purity > 98%) as internal standard was obtained from Polymer Source (Toronto, Canada). Potassium hexaciyanoferrate trihydrate, hexane, zinc sulfate heptahydrate, formic acid, and hydrochloric acid were supplied from Merck (Darmstadt, Germany) and acetonitrile (HPLC grade) was from Lab-Scan (Dublin, Ireland). HPLC grade water was supplied from VWR (EC). Standard stock solutions of acrylamide and D_3-AA were prepared by dissolving the chemicals in acetonitrile. These stock solutions contained 250 µg of acrylamide and 250 µg D_3-AA in 1 mL, separately. For the solid phase extraction OASIS MCX cartridge (3 cm^3/60 mg, Waters, Milford, Massachusetts, USA) was used.

Methods

Acrylamide Analysis

The method developed by Alpözen and Üren [1] and Alpözen et al. [2] was used for acrylamide analysis. According to the developed method 0.025, 0.05, 0.125, 0.25, 0.625, 1.25, and 2.5 mL of 1 mg/L acrylamide solution were taken and 2 mL of 1 mg/L D_3-AA solution and 20 mL of distilled water were added. Then pH of the solutions was adjusted to 2.3 by hydrochloric acid and diluted to 25 mL with distilled water. Final concentration of D_3-AA in all these solutions was 80 µg/L. Six grams of sample was homogenized in 30 mL of distilled water, suspension was taken into a 100 mL conical flask and shaken for 30 min. The mixture was clarified by 1.5 mL Carrez I (23% w/v, $ZnSO_4.7H_2O$) and 1.5 mL Carrez II (15% w/v, $K_4Fe(CN)_6.3H_2O$) solutions and then the mixture was centrifuged at 10400 g for 15 min. The supernatant was collected and defatting was performed by using 20 mL of hexane. Twenty mL of the aqueous phase was taken and 2 mL of 1 mg/L D_3-AA was added. This sample was treated with hydrochloric acid to obtain a pH of 2.3 and diluted to 25 mL with distilled water. Waters OASIS MCX SPE cartridge (New York, USA) was used for further clean-up. The pass through strategy for the SPE clean-up was applied to retain the matrix interferences [8]. OASIS MCX cartridge was conditioned consecutively with 1 mL of methanol and 1 mL water at a rate of one drop per second. Air was passed through the cartridge by pushing injector to remove the remaining water. 1 mL of extract was passed through the cartridge at a rate of one drop per second. The first 10 drops of eluate were discarded while the remaining drops were collected in a vial and 15 µL of the sample was injected to LC-MS/MS. Analysis was performed in duplicate.

Chromatographic Conditions

Mobile phase was prepared by mixing solvent A and solvent B in a ratio of 90:10 (v/v), respectively. Solvent A was 0.3% (v/v) aqueous formic acid solution and solvent B was acetonitrile. The analytical separation was performed on a Zorbax column C_{18} (50 mm x 4.6 mm,

1.8 µm, Santa Clara, USA) using the isocratic mixture at a flow rate of 0.8 mL/min at 25°C with an injection volume of 15 µL. Stop time was 2 min. For the optimization of chromatographic conditions, injection volume was evaluated and increased to 15 µL to obtain higher signals. Different mobile phase compositions were examined and by acidifying the mobile phase, signal values were also increased.

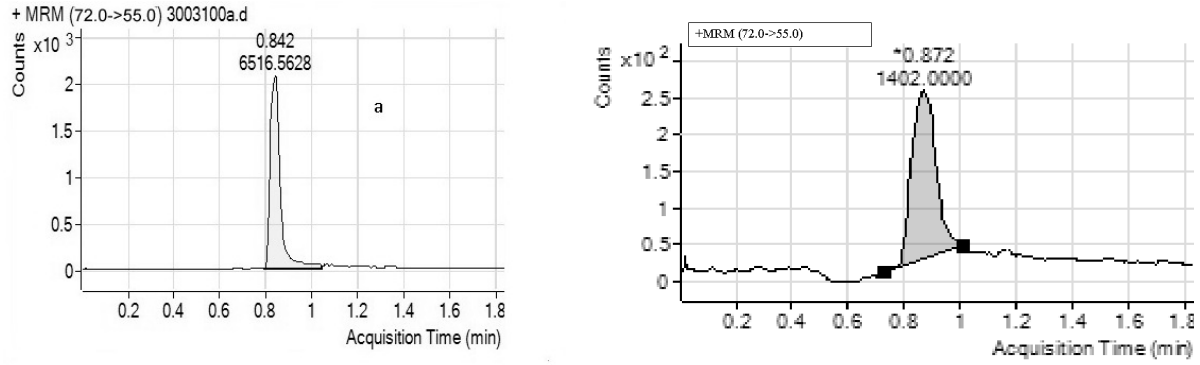
Apparatus: Chromatographic separations were performed by using Agilent 1200 liquid chromatograph (Santa Clara, USA) connected to 6410 triple quad MS-MS detector with electrospray ionization in the positive-ion mode, equipped with a quaternary pump, an autosampler, and a temperature-controlled column oven. Data acquisition was performed in selected ion monitoring (SIM) mode. Multiple reaction monitoring (MRM) of transition ions was m/z 72.0→55.0 for acrylamide and m/z 75.1→58.0 for D_3-AA. For identifying acrylamide m/z 72.0→54.0 ion was also monitored. The optimized MS instrument parameters were drying gas temperature (N_2) of 350°C with a flow rate of 12 L min^{-1}, nebulizer pressure of 275.8 kPa, capillary voltage of 4 kV, fragmentor voltage of 60 V for D_3-AA and 70 V for acrylamide, collision energy of 10 V for each transition. Fragmentor voltage was evaluated for acrylamide and D_3-AA determination and optimum conditions were determined as given above. The concentration of acrylamide in bread samples was determined by a calibration curve which was daily prepared by plotting peak area ratios of the analyte acrylamide i.e., 55 to the internal standard D_3-AA i.e., 58 versus amount of acrylamide injected.

Statistical Analysis

Statistical analyses were realised with the SPSS 20.0 statistics package programme.

RESULTS and DISCUSSION

An accurate, practical, and non-toxic LC-MS/MS method was used for trace determination of acrylamide in light biscuit. The chromatograms of fragment ion m/z 55.0 for acrylamide in standard solution and light biscuit sample are demonstrated in Figure 1. Certified reference materials of crisp bread with T-3021 and T-3026 reference codes supplied by FAPAS (UK) were analysed. Acrylamide contents of these samples were found in the range cited in the certificates.



a b

Figure 1. LC-MS/MS chromatograms of acrylamide fragment ion m/z 55.0 a) in standard acrylamide solution (100 µg/L) b) in light biscuit samples

Concentrations of acrylamide in 10 light biscuit samples from different manufacturers are shown in Table 1. As is seen in the table, acrylamide contents of light biscuit samples varied from 42.57 µg/kg to 877.57 µg/kg with an average content of 394.47 µg/kg. This difference may be resulted from formulation of biscuit, biscuit production stages, baking time and temperature, sugar and protein content of raw materials used biscuit.

This work is the first survey study for the determination of acrylamide contents of different light biscuits produced in Turkey by LC-MS/MS method.

There is no limit for acrylamide levels of food in legislation of European Union Countries and our country. But, limit of 00.1 µg/L was determined for water in Europe and in our country [3]. Europe Food Safety Authority and Ministry of Food, Agriculture and Livestock continue studies on determination of acrylamide levels in different food samples.

Table 1. Acrylamide content of light biscuit samples

Sample Number	Acrylamide Level (µg/kg)
1	$444.68^{cd} \pm 23.49$
2	$877.57^{a} \pm 18.02$
3	$433.36^{d} \pm 17.39$
4	$229.03^{f} \pm 14.33$
5	$42.57^{h} \pm 5.95$
6	$467.06^{c} \pm 8.15$
7	$694.75^{b} \pm 4.07$
8	$382.28^{e} \pm 7.84$
9	$231.20^{f} \pm 4.83$
10	$142.22^{g} \pm 2.65$

[a]Different matching letters in a column mean significant differences according to Duncan test ($p<0.05$)

CONCLUSION

A rapid and reliable LC-MS/MS method was used for the determination of acrylamide in light biscuit. The method was linear up to 750 µg/kg food with a determination coefficient of 0.999. Recovery rate was found as 99.3% with limit of detection and limit of quantification values of 1.5 µg/kg and 5.0 µg/kg, respectively. With this study, acrylamide levels of biscuit samples produced in Turkey were determined. These results will be important data for determining limits of biscuits for the Ministry of Food, Agriculture and Livestock.

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