

Acrylamide Contents of Some Commercial Crackers, Biscuits and Baby Biscuits

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ABSTRACT

In present study, acrylamide levels in a total of 90 commercial samples of crackers, biscuits and baby biscuits sold in Turkey were determined by the GC/MS method with bromine derivatization. Cracker samples (n=30) were divided into 4 groups as salty crackers, crackers with cheese, crackers with spices and crackers with sesame. Biscuit samples (n=27) were divided into 5 categories as wheat based biscuits, bran based biscuits, whole wheat biscuits, oat biscuits and wheat biscuits with cocoa. Baby biscuits (n=33) were grouped depending on their brand name. Mean acrylamide levels were 604, 495 and 153 µg/kg for crackers, biscuits and baby biscuits, respectively. Acrylamide contents showed a great variation among different brands and types of food samples since acrylamide can be found in a wide range of food products at different levels depending on the composition and processing parameters.

Keywords: Acrylamide, GC-MS, Thermal processing contaminants, Biscuits, Crackers

Bazı Ticari Kraker, Bisküvi ve Bebek Bisküvilerindeki Akrilamid Miktarları

ÖZ

Bu çalışmada, Türkiye’de satılan 90 adet ticari kraker, bisküvi ve bebek bisküvisi örneklerindeki akrilamid düzeyleri brom türevlendirmesi sonrasında Gaz Kromatografisi/Kütle Spektrometresi metoduyla araştırılmıştır. Kraker örnekleri (n=30) tuzlu, peynirli, baharatlı ve susamlı krakerler olmak üzere 4 gruba ayrılmıştır. Bisküvi örnekleri (n=27) ise 5 gruba ayrılarak sade, kakaolu, kepekli, tam buğday ve yulafli bisküviler olarak sınıflandırılmıştır. Bebek bisküvileri (n=33) markalarına göre gruplandırılmışlardır. Kraker, bisküvi ve bebek bisküvisi örneklerindeki ortalama akrilamid düzeyleri sırasıyla 604, 495 ve 153 µg/kg olarak belirlenmiştir. Akrilamid gıdaların kompozisyonuna ve işleme parametrelerine bağlı olarak pek çok gıdada bulunmakta ve düzeyleri farklı markalara ve gıda çeşitlerine göre değişmektedir.

Anahtar Kelimeler: Akrilamid, GC-MS, Isıl işlem kontaminantları, Bisküvi, Kraker

INTRODUCTION

Acrylamide (CH₂=CH-CONH₂) is a colorless, odorless and water soluble (215.5 g/100 mL at 30°C) crystalline chemical compound (CAS Registry Number 79-06-1) with a molecular mass of 71.08 g/mole [1, 2]. It is an important industrial chemical used in the production of

polyacrylamide, which is used in electrophoretic separation, water treatment and paper processing [2-4]. Before detection in foods, the main concern was occupational exposure or at low levels non-occupational exposure as a result of the migration of acrylamide from food packaging material or from water through water treatment [5]. In April 2002, acrylamide was detected in

carbohydrate rich foods processed at high temperatures and discovery of acrylamide in foods attracted considerable attention worldwide since it is classified as probably carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer [6].

People are exposed to acrylamide by diet as a result of the consumption of acrylamide rich food products. The Maillard reaction between reducing sugars and free asparagine during high temperature processing was reported as the main and the most probable pathway for the formation of acrylamide in foods [7-9]. Therefore, acrylamide can be found at different levels in carbohydrate rich foods heated at high temperatures during their production and processing such as potato products, cereal products and roasted coffee [10, 11]. Cereal products including biscuits, baby biscuits, breads, crackers and breakfast cereals contain acrylamide at various levels. Ölmez et al. [12] reported an overview of acrylamide contents in a total of 311 processed and traditional Turkish foods. Results of this study showed that mean acrylamide levels in crackers (n=18), biscuits (n=16) and baby biscuits (24) were 247, 198 and 152 µg/kg, respectively. Şenyuva and Gökmen [13] showed acrylamide levels in 120 analyzed food samples taken randomly from markets in Turkey. In this study, the highest mean acrylamide level reached to 1072 µg/kg in crackers. In a study by Pacettia et al. [14], acrylamide levels in selected Colombian foods (n=112) were determined and bakery products like biscuits (1104 µg/kg), showed the highest mean acrylamide value. In another study, acrylamide contents in commercial biscuits and bread derivatives marketed in Spain were investigated [15]. In biscuits, mean and highest acrylamide contents were 423 and 2085 µg/kg, respectively. Biscuits and crackers may contain high acrylamide levels, and these foods might pose serious public health risks since these foods are widely consumed by people especially children. It was reported that acrylamide exposure of children is higher than that of adults [16-18]. Mean acrylamide exposure levels in Europe were estimated by the European Food Safety Authority (EFSA) as 0.31-1.1, 0.43-1.4, 0.70-2.05 and 1.2-2.4 µg/kg body weight (bw)/day for adults (>18 years), for adolescents (11-17 years), for children and for toddlers (1-3 years), respectively [11]. It is apparent from the reported data that exposure of toddlers is in the highest range.

In a previous work of ours, exposure of the toddlers was estimated as 1.43 µg/kg bw/day and following bread, the food products that contributed to the acrylamide exposure the most were crackers, biscuits and baby biscuits accounting of the 25, 19 and 11 % of the total dietary intake of acrylamide by toddlers [19, 20]. These food products were major sources of dietary acrylamide exposure due to their relatively high consumption rate and/or high acrylamide levels [11, 17, 18, 20-29]. Therefore, exposure to acrylamide is of concern for consumers due to its potential carcinogenicity and content of acrylamide in commonly consumed food products must be determined by highly sensitive methods. In the literature, a great number of methods have been reported for analyzing acrylamide levels of

the food products. These methods are mainly based on mass spectrometry as the determinative technique coupled with liquid chromatography or gas chromatography [14, 15, 30-35].

The aim of this present study was to determine the contents of acrylamide in crackers, biscuits and baby biscuits of different brands and types which are commonly consumed in Turkey by GC/MS after bromination.

MATERIALS and METHODS

Chemicals and Standards

Acrylamide standard (>99%), the internal standard ¹³C₃-acrylamide (1,2,3- ¹³C₃ - acrylamide, >99%) and the acrylamide derivative (2,3 dibromopropionamide >99.5%) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany), Cambridge Isotope Laboratories Inc. (Andover, USA) and Chem Service Inc. (West Chester, USA), respectively. Ethyl acetate was purchased from Sigma-Aldrich (St. Louis, USA). Other chemicals were used of brand Merck KGaA (Darmstadt, Germany). Water was produced by an ultrapure (18.2 MΩ cm at 25°C) purification system (Bedford, USA). Food products were randomly collected from five different supermarkets in Antalya in 2012.

Sampling

Widely consumed brands and types of crackers, biscuits and baby biscuits samples were selected for the study. A total of 90 samples were analyzed to determine the acrylamide levels. Samples were categorized to sub-groups depending on their brand and/or composition. Cracker samples (n=30) were grouped as salty crackers (15), crackers with cheese (4), crackers with spices (6) and crackers with sesame (5). Biscuit samples (n=27) were divided into 5 categories as wheat based biscuits (18), bran based biscuits (4), whole wheat biscuits (2), oat biscuits (1) and wheat biscuits with cocoa (2). Baby biscuits (n=33) were grouped depending on their brand and number of samples for each group were ranged from 4 to 9.

Sample Extraction and Bromination

Before extraction, LOD (limit of detection) and LOQ (limit of quantification) values were estimated as three times and ten times the standard deviation, respectively. For the recovery test, six replicates were conducted with two different concentrations (at a final concentration of 0.3 mg/kg and 1 mg/kg) by spiking a baby biscuit sample with ¹³C₃-internal acrylamide standard.

Sample extraction and bromination was carried out according to a procedure reported previously [36, 37]. Ground samples defatted by *n*-hexane (1:1) and 1 g of defatted sample was suspended in 8.2 mL of water (60°C). Suspension was spiked with 200 µL ¹³C₃-internal standard (15 mg/L) and stirred on a magnetic heater stirrer. Proteins were removed by using Carrez clearing agents. This mixture was centrifuged and then aqueous

layer was filtered through a 0.45 µm syringe filter. For the derivatization, 300 µL of bromine solution (15.2 g of potassium bromide, 0.8 mL of hydrobromic acid, 5 mL of 1.6% saturated bromine water and 60 ml of distilled water) was added to the clarified solution. The reaction mixture was transferred into an ice bath to allow the reaction in the dark. Approximately an hour later, reaction was completed and excess bromine was decomposed by adding sodium thiosulfate solution. Then 2 mL of ethyl acetate was added, and the tubes were centrifuged at 5000 rpm for 10 minutes. The upper phase was transferred into a GC-MS vial and triethylamine (1:10) was added to convert the acrylamide derivative, 2,3 dibromopropionamide (2,3-DBPA), to a more stable derivative, 2-bromopropionamide (2-BPA), prior to analyses.

GC-MS Conditions

A Thermo Scientific ISQ GC-MS system (Thermo Fisher Scientific Inc. Waltham, MA, USA) equipped with a fused capillary column (TR-WAX, 30 m x 0.25 mm x 0.25 µm) was used in acrylamide analysis. The oven temperature program was as follows: A 50°C initial temperature was held for a minute, then increased to 180°C at 20°C/min, then to 260°C by a rate of 10°C/min, and held for 10 min at this temperature. The injection block, detector and ion source temperatures were 240, 250 and 230°C, respectively. Carrier gas (helium) flow through the column was 1 mL/min. Injection volume was 2 µL and identification was determined using Selective Ion Monitoring (SIM) mode.

Statistical Analysis

All statistical analyses were performed using the SPSS 17 (Statistical Package for the Social Sciences) software. Statistical significance was considered at $p < 0.05$.

RESULTS and DISCUSSION

In our previous works, acrylamide levels of different food products were determined by adapting an extraction, GC-MS and bromination method, and then these values were used to calculate the acrylamide intake and exposure of toddlers [19, 37]. In present study, a total of 90 samples including crackers, biscuits and baby biscuits were analyzed by categorizing the food products into sub-groups. LOD and LOQ values were estimated to be 7.46 µg/kg and 24.88 µg/kg, respectively. The LOQ value was less than the maximum recommended LOQ values of the analytical methods should meet for foods of infants and young children for cereal products as 30 and 50 µg/kg, respectively as reported by European Commission [38, 39]. Recovery was 83% that agrees with those obtained in previous studies [40, 41]. Acrylamide contents of 90 commercial samples ranged from below the LOQ to 2666 µg/kg depending on the type of products. Acrylamide contents of the commercial samples are shown in Table 1. Mean acrylamide levels of the crackers, biscuits and baby biscuits were determined as 604, 495 and 153 µg/kg, respectively. Crackers were

divided into 5 categories and the highest acrylamide levels among the sub-groups of the crackers were determined in the crackers with spices reaching to 2666 µg/kg. Mean acrylamide contents in all crackers types were aligned from high to low as crackers with spices (1376 µg/kg) > salty crackers (524 µg/kg) > crackers with sesame (339 µg/kg) > crackers with cheese (76 µg/kg). Biscuits were grouped depending on the type of products. Mean acrylamide contents of biscuits were 337 µg/kg, 633 µg/kg, 755 µg/kg and 923 µg/kg for wheat based biscuits, whole wheat biscuits, bran based biscuits and wheat biscuits with cocoa, respectively. Only a sample was analyzed in the category of oat biscuits and acrylamide level of this product was almost the highest among the biscuits reaching to 1153 µg/kg. Acrylamide levels in baby biscuits were lower than the biscuits and crackers. Acrylamide contents of different brands of baby biscuits were aligned from high to low as brand 1 baby biscuits with banana (302 µg/kg) > brand 3 baby biscuits (265 µg/kg) > Brand 3 baby biscuits with banana (129 µg/kg) > Brand 1 baby biscuits (123 µg/kg) > Brand 2 baby biscuits (67 µg/kg).

There were statistically insignificant differences between mean acrylamide levels of crackers and biscuits ($p > 0.05$). On the other hand, it was observed that differences in the acrylamide contents of baby biscuits were statistically significant at the level of 5% when they were compared to the acrylamide levels of biscuits and crackers (Table 1).

Figure 1 shows how many samples in the ranges of <LOQ, LOQ–200, 200–400, 400–600, 600–800, 800–1000 1000–1200 and 1200 < µg acrylamide/kg sample to show the different acrylamide levels and variation between the samples. Acrylamide levels were the highest in crackers among the food groups and reached to above 2000 µg/kg in some cracker types. Biscuits followed to crackers and in some types acrylamide levels reached to above 1000 µg/kg.

In other studies on acrylamide levels of food products in Turkey, high levels of acrylamide were reported in crackers and biscuits that were in agreement with our results [12, 13]. Şenyuva and Gökmen [42] reported the acrylamide levels in totally 30 crackers and biscuits samples as 1072 and 389 µg/kg, respectively. In baked cereal based foods, acrylamide is formed as a result of the high temperature baking. Therefore, acrylamide was reported at different levels in biscuits and crackers [14, 43, 44]. In present study, wide variation in the acrylamide contents of different brands and types of food groups were observed as indicated from the high levels of standard deviation values. Similar findings were obtained by other researchers [12, 13, 45]. This variation can be as a result of the different composition and processing conditions in different brands and types of food samples since acrylamide formation is directly related to these factors. Differences in the raw material composition such as free asparagine and reducing sugar content, food product formulations, processing methods and parameters such as pH, water content, high temperature (more than 120°C) and time could be the sources for variation in acrylamide levels as reported

by other researchers [7, 8, 45-57]. Crackers with spices were the sub-group of crackers containing appreciably high levels of acrylamide with mean and the highest values of 1376 and 2666 mg/kg, respectively. Differences in the mean acrylamide levels of the crackers with spices were statistically significant when they were compared to the other types of crackers as it can be seen in Table 1. Since the formulation was not known completely, it is not possible to discuss the effects of ingredients. But it is known that these types of crackers contain some spices, such as pepper, and these spices can positively or negatively affect the

acrylamide formation. In the literature, there are some studies evaluating the impact of spices on acrylamide formation. But these studies were focused on the antioxidant effect of the spices to reduce the acrylamide levels in different matrixes such as cakes and potatoes and some of the spices decreased the acrylamide formation while some of them increased or did not have any effect on acrylamide levels [58, 59]. But crackers with spices include different constituents depending on the brand and need to be further assessed from the ingredients point of view to discuss the effects of spices in details.

Table 1. Acrylamide levels in commercial crackers, biscuits and baby biscuits ($\mu\text{g}/\text{kg}$)

Food Product	n ¹	Mean \pm SD ²	Range	Subgroup	n ¹	Mean \pm SD ²	Range
Crackers	30	604 ^a \pm 694	<LOQ- 2666	Salty crackers	15	524 ^b \pm 457	<LOQ- 1251
				Crackers with cheese	4	76 ^b \pm 153	<LOQ- 307
				Crackers with spices	6	1376 ^a \pm 1035	566-2666
				Crackers with sesame	5	339 ^b \pm 344	<LOQ- 814
Biscuits	27	495 ^a \pm 403	<LOQ- 1177	Wheat based biscuits	18	337 ^b \pm 352	<LOQ- 1072
				Bran based biscuits	4	755 ^{ab} \pm 374	344-1097
				Whole wheat biscuits	2	633 ^{ab} \pm 143	532-735
				Oat biscuits	1	1153 ^a \pm 0	1153
				Wheat biscuits with cocoa	2	923 ^{ab} \pm 359	669-1177
Baby biscuits	33	153 ^b \pm 201	<LOQ- 588	Brand 1 baby biscuits	9	123 ^a \pm 245	<LOQ- 588
				Brand 1 baby biscuits with banana	4	302 ^a \pm 202	377-433
				Brand 2 baby biscuits	9	67 ^a \pm 110	<LOQ- 306
				Brand 3 baby biscuits	5	265 ^a \pm 198	<LOQ- 548
				Brand 3 baby biscuits with banana	6	129 ^a \pm 205	<LOQ- 539
				Total	90	406 ^b \pm 508	<LOQ- 2666

Different letters indicate significant differences among food products and among sub-groups independently (Duncan (0.05), within same column). ¹: number of samples; ²: standard deviation

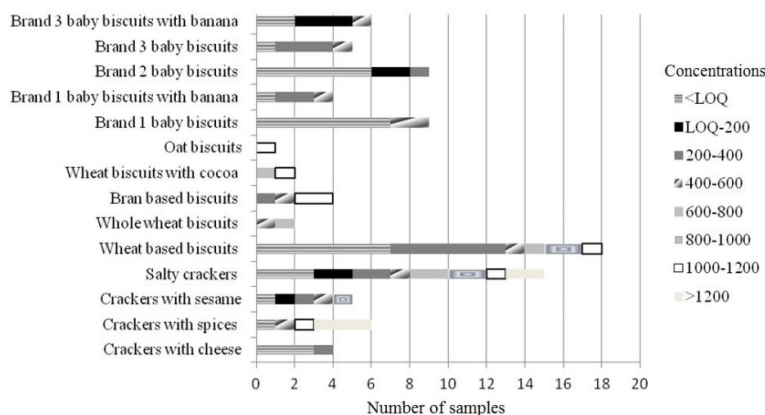


Figure 1. Number of samples in various ranges of acrylamide ($\mu\text{g}/\text{kg}$)

Acrylamide level of biscuits was the lowest in wheat based biscuits in comparison to other biscuits (Table 1). Asparagine is an important precursor for the formation of acrylamide since it is the main amino acid that reacts with reducing sugars to produce acrylamide as it was reported [60]. Acrylamide concentration of bakery products can change depending on the type of flour used in the production [61]. It is known that free asparagine content of the sifted wheat flour is less than the whole grain flours [46]. As a result of that, acrylamide contents of wheat based biscuits were 337

$\mu\text{g}/\text{kg}$ that was lower than other biscuits types in the present study. This finding was in consistent with our previous study that reported the lower acrylamide levels of wheat breads compared to the other bread types [36]. In the present study, acrylamide levels of the bran based biscuits were higher than the wheat and whole wheat based biscuits. This result is in consistent with the finding of high free asparagine content in bran when it is compared to other parts of the cereal grain [46]. Taeymans et al. [45] also reported that addition of whole wheat flour and bran to biscuit formulas tended to

increase acrylamide in comparison with plain counterparts. In the present study, the highest acrylamide levels in biscuits were determined in the oat biscuits. It was reported that free asparagine content of the oat flour is high and result of the present study is in consistent with that finding [62]. Cocoa beans contain free asparagine and roasting process increases acrylamide in cocoa [63]. Results of our study showed that wheat biscuits with cocoa also contained important levels of acrylamide reaching to 1177 µg/kg.

This present study analyzed 33 baby biscuits samples including different brands and types commonly consumed by toddlers in Turkey and mean acrylamide levels of baby biscuits were 153 µg/kg, which was in agreement with previous studies [12, 25]. However, there were not any significant differences in the acrylamide levels of baby biscuits belonging to different brands and types. Higher levels of acrylamide in baby biscuits samples were reported by other researchers from different countries reaching to 1217 µg/kg with the mean value of 324 µg/kg [23]. The mean acrylamide content in baby biscuits determined in our study was also lower than those of reported in Poland (219 µg/kg) [29]. The highest amount revealed in baby biscuits in a single sample (588 µg/kg) was lower than that found in Germany (633 µg/kg) [17]. EFSA reported acrylamide amounts of biscuits and rusks for infants as a sub-group in the monitoring study of 2010 in Europe as 86 µg/kg [43].

Results of our study showed that baby biscuits contain lower acrylamide when they are compared to biscuits and crackers. Exact formulation and processing parameters are not known completely, therefore, it is not easy to discuss the differences in acrylamide levels. But if we compare wheat biscuits and crackers with baby biscuits, because baby biscuits that were bought in our study were made of wheat instead of other cereals, wheat biscuits and crackers are thinner as they are observed. In the study of Açar and Gökmen [64], crust-like model was developed, and it was reported that the product thickness significantly influence acrylamide formation rate during baking. Therefore, lower acrylamide values in baby biscuits can be related to its thickness. But there is need for more studies to discuss the acrylamide formation in different food products by developing models.

CONCLUSIONS

This research reports the acrylamide contents of commercial crackers, biscuits and baby biscuits obtained from Turkish market. The mean acrylamide contents of these products ranged from below the LOQ to 2666 µg/kg. The results revealed that acrylamide contents of different brands and types of samples show a wide variation as indicated from the high levels of standard deviation. Composition and processing conditions play an important role in acrylamide formation, as different ingredients have various amounts of free asparagine and reducing sugars available for the reaction for the formation of acrylamide. Therefore, different amounts of acrylamide may be present in a

wide range of food products depending on the ingredients and processing parameters. The food product groups that were chosen for the present study are commonly consumed foods by people especially children. Exposure of children is higher than adults as a result of the lower body weight of children and also high consumption rate of crackers and biscuits. As a result of that, mitigation strategies in commercial samples must be a high priority to decrease acrylamide exposure levels.

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