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Investigation of acrylamide levels in branded biscuits, cakes and potato chips commonly consumed in Pakistan

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Abstract

The harmful effects of toxic chemical contaminants such as acrylamide in food pose a major health problem in the human being. The presence or absence of residues of chemical toxic contaminants in food products has main implications in respect to both food protection and international trade. Carcinogenic acrylamide can be produced by heat treated carbohydrate foods in high levels such as fried and baked products. The aim of these studies were to investigate carcinogenic acrylamide levels in potato chips, branded biscuits and cakes which are commonly consumed in Pakistan by using state of the art instrument, gas chromatography mass spectrometry. Most of biscuits and potato chips products contain high amount of acrylamide content except cakes. These studies are the major source to establish the data about acrylamide in different selected food of different companies and give an overview about the acrylamide situation and health hazard of branded biscuits, cakes and potato chips in Pakistan. The levels of acrylamide in biscuits found in the range of 52.3 ± 0.70 to 507.5 ± 1.5 µg/kg; but in cakes it was low while in potato chips 27.1 ± 0.65 to 1323.0 ± 3.0 µg/kg. The acrylamide level is reported first time in Pakistani food products. Acrylamide formation in baked and fried products is still of concern, as the formation of acrylamide in food products of developing country is still unknown for consumers and industries. Therefore it is an urgent need for reducing the level of acrylamide produced during food processing. These studies are provided interesting and valuable results for the development of mitigation strategies by the Pakistani industries.

Introduction

Safety of food products has great importance for health. Acrylamide has been found in an extensive variety of food products. It is the most prevalent in potato chips, French fries, fried food, roasted cereals, coffee, tea and baked products, which is produced during frying and baking processes by ignoring the frying conditions and other relevant variables which formed a dangerous chemical compound called acrylamide which can cause severe health problems (Matthäus *et al*. 2004; Pedreschi *et al.* 2006; Gokmen *et al*. 2006; Liu *et al.* 2008; Ye *et al*. 2011; Yuan *et al.* 2011). In 1994, the International Agency for Research on Cancer (IARC) classified acrylamide as potentially carcinogenic to human. (IARC, 1994). The nervous system in humans and animals can be damaged by the exposure of acrylamide and *in vitro* and *vivo* experimental systems confirmed that acrylamide is a mutagenic and carcinogenic reproductive toxin (Dearfield *et al*. 1988; Costa *et al.* 1992; Lopachin and Lehning 1994; Dearfield *et al*. 1995). Human beings can be exposed directly to it by consuming high temperature treated foods rich in carbohydrate, such as baked foods, fried foods, or ultimately through certain packaging materials. It is concluded by the characterization of acrylamide that it has been strengthened cancer risk for humans (FDA/WHO 2002; Samuelson, 2003; Matthäus *et al.* 2004; Zhang *et al.* 2005; Husamo *et al*. 2010; Aladedunye *et al*. 2011). In 2000, Tareke *et al.* demonstrated the importance of acrylamide in food first time (Tareke *et al.* 2000). In 2002, the Swedish National Food Administration (NFA) reported about the presence of acrylamide in baked, fried foods, cereal products and coffee (Tareke *et al.*, 2002; Surdyk *et al*., 2004). The Swedish National Food Administration in 2005 announced that foods processed and cooked at elevated temperatures have relatively high content of acrylamide (Zhang *et al.*, 2005). It is reported that human are exposed by acrylamide from 20% bakery and 50% potato products (Erickson, 2000). The toxic effects produced by oral doses of >100 mg/kg of body weight and medium lethal doses (LD_{50})

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are >150 mg/kg of body weight (JECFA, 2006). The following factors are affected in the formation of acrylamide in foods; such as processing conditions (i.e. temperature, moisture content, baking time and matrix of products) and acrylamide precursors are reducing sugar, free amino acids (principally asparagine).

Acrylamide is mostly formed by involving some amino acids and reducing sugar at elevated temperature or during the course of maillard reaction (Eriksson, 2005; Keramat *et al.*, 2011). The alternate way an amino acid reacted with a dicarbonyl compound by lose of water and followed to intra-molecular rearrangement of ion and strecker degradation to form an acrolein, a hydrogen nitride and strecker aldehyde or other compounds (Mottram *et al.*, 2002). Acrylamide was also produced by the reaction of acrylic acid and hydrogen nitride or ammonium originated from nitrogen containing compounds (Eriksson, 2005).

A great interest for the discovery of acrylamide in food consumption prompted to investigate the levels of contamination of acrylamide in selected food stuffs. Fried and baked foods are widely consumed all over the world. Potato chips, biscuits and cakes are prevailing foods and almost consumed every day in Pakistan. Potato chips and biscuits are considered one of the favorite foods for children and adults which are mostly consuming in the school lunch and evening time. Regarding the importance of acrylamide formation in these groups of food, this study, therefore, aims at investigating the acrylamide content in potato chips, biscuits and cakes.

This knowledge would be beneficial for industries in Pakistan to improve the effect of food processing steps to decrease the formation of acrylamide in food products. This is the first paper reporting the occurrence of acrylamide in Pakistani food products such as in different varieties of potato chip, biscuits and cakes.

Materials and Methods

Samples

The samples of potato chips (16 samples; PC-1 – PC-16), branded biscuits (16 samples; BB-1 – BB-16) and cakes (03 samples; C-1 – C-3) in 20 gm packing of different companies were purchased from different supermarkets of Karachi, Pakistan each in triplicate. The choice of the brands was based on the highest consumption among those available in the market. The sample packets were wrapped properly in air tight plastic bags and transported by air in one day from Pakistan to Germany and kept in laboratory frieze until analysis.

Chemicals

Petroleum ether, ethyl acetate, water (HPLC grade), potassium hexacynoferrate (II) trihydrate (37.5 g/250 ml; Carrez I), zinc sulfate hepta hydrate (75 g/250 ml; Carrez II), sodium chloride, and methanol were purchased from Merck, Darmstadt, Germany. Standard acrylamide-2,3,3-d3 (98%) was purchased from Cambridge Isotope Laboratories, Andover, MA, USA.

Standard Solutions: Stock solution of 150 µg/ml of acrylamide-2,3,3-d3 was prepared with HPLC grade water.

Extraction method of acrylamide from food samples

After extraction of acrylamide from biscuits, chips and cake samples, it was determined by a Gas Chromatography Mass Spectrometry method in the EI mode. Deuterated acrylamide was used as an internal standard for quantification. Briefly, 15 g of the sample was grinded in a labor mill (Grindomax GM 200, Retsch, Haan). Weigh 4.00 g of grinded sample and added 50 µl of internal standard (150 µg/ml) and extracted with 50 ml bi-distilled water at 60^{o}C for 30 mins by ultrasonically. This extract was centrifuged at 25000x g (+10 - +20^{o}C) and the supernatant solution was defatted by petroleum ether. The solution was clarified by 5 ml Carrez I and II solution, each. The solution was centrifuged again to separate precipitate and then acrylamide was salted out with sodium chloride. Acrylamide was extracted 3 fold with 30 ml ethyl acetate. These organic phases were filtrated by water repellent filters (MN616 wa ¼, Macherey-Nagal GmbH & Co. KG, Duren, Germany) to separate water from EtOAc phase. The organic phase containing acrylamide was evaporated to about 0.5 ml before using for capillary gas chromatography-mass spectrometry (Matthäus *et al.,* 2004).

Determination of acrylamide by Gas Chromatography-Mass Spectrometry

For the determination of acrylamide and D3-acrylamide, gas chromatography mass spectrometry analysis was carried out using the electron ionization mode (EI, 70 eV) on a Hewlett Packard instrument Model 5890 Series II. The identification was achieved by the ions with masses (m/z) 55, 58, 71 and 74 by using SIM-technique (Selected Ion Monitoring). The quantification was carried out by ions with masses 71 and 74. The separation was achieved with a DB-23 capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness; J & W Scientific Products GmbH, Köln, Germany). The carrier gas was helium at a flow rate of 1.0 ml/min. The column temperature was initially kept at 80°C for 2 mins., then increased from 80°C – 220°C at rate of 10°C/min. The final temperature 220°C was hold for 1 min. Other operating conditions were a split/splitless injector (splitless, temperature 240°C, and an ion source temperature is 200°C). Quantification and identification of acrylamide in the products was determined by internal standard of deuterated acrylamide and by comparison of retention times and mass spectra of known standard. The analyses were carried out in triplicate and expressed as mean ± standard deviation (SD) which was calculated by Microsoft excel.

Results and Discussion

The preparation of sample is tricky for the analysis of acrylamide. It is difficult to handle for such kind of samples which have low level of acrylamide, it is necessary to work with precautions during all steps of extractions. 150 µg/ml of deuterated acrylamide was spiked into blank and food samples and extracted in the same manner as describe in the sample preparation (experimental section). Finally acrylamide was migrated from aqueous phase to organic EtOAc (ethyl acetate) phase; EtOAc was evaporated under vacuum and residue was re-dissolved in 0.5 ml ethyl acetate for analysis.

For the quantification and detection of acrylamide, the electron ionization mode was applied in gas chromatography and mass spectrometry, the main fragment ions are appeared at m/z 55 and 71, respectively. These ions are used for quantification. The quantification is performed by adding isotope labeled acrylamide as internal standard. The LOD can be 10 – 50 µg/kg. The standard addition method is a precise quantification method and it is useful for all type of sample (Ito and Tsukada, 2001; Mottram, 2002; Basilicata et al., 2005; Keramat *et al*., 2011).

The acrylamide level in the food products of Pakistan was determined first time. The presence of acrylamide in food products should be alarmed food industries and food control authorities. The acrylamide levels in potato chips, branded biscuits and cakes are depicted in table-1. Total sixteen samples of branded biscuits (BB-1 to BB-16) and three samples of cakes (C-1 to C-3) were evaluated and the values are depicted in table-1. The acrylamide was found in all of the branded biscuit samples in the range of 52.3 ± 0.70 to 507.5 ± 1.5 µg/kg. According to European commission the indicative value for biscuits are 500 µg/kg, which is comparable with biscuits results (EFSA, 2012). But the permissible value of acrylamide are as follows for different commissions; FAO/WHO (0.3 – 0.8 µg/kg-bw /day), BfR, Germany (1.1 - 3.4 µg/kg-bw /day), SNFA Sweden (0.45 – 1.03 µg/kg-bw /day), USA (0.43 – 2.31 µg/kg-bw /day), UK (0.3 – 1.8 µg/kg-bw /day) and JECFA (1 – 4 µg/kg-bw /day) (EFSA, 2012).

Cakes consist of less amount of acrylamide in the range of (traces – 35 ± 0.15 µg/kg). The high content of acrylamide just depends on the raw material used and the most important is the baking and formulation condition of dough. As the use of reducing sugar in the formulation with high carbohydrate that will definitely increase the acrylamide content at high temperature.

The reason could be attributed to the ingredients of mixtures used in the preparing and processing of biscuits and cakes. The level of acrylamide depends on the proportion of carbohydrate which increases the acrylamide level. Also the ingredients may contain citric acid or any other material such as sodium bicarbonate which has a role in changing pH that affects the acrylamide level (Eriksson, 2005).

Sixteen different varieties of potato chip samples PC-1 to PC-16 (plain chips, rings, top pops, balls, slanty and spicy chips) were evaluated for the acrylamide level and found in the range of 27.1 ± 0.65 to 1323.0 ± 3.0 µg/kg (Table-1). Acrylamide contents in potato chips were reported 752 and 117 – 2762 µg/kg by JECFA in 2006 and FDA in 2002, respectively but not comparable with the estimated dietary intake of FAO/WHO set value of acrylamide 0.3 – 0.8 µg/kg-bw/kg (FDA 2002; JECFA, 2006). The difference in the results of two different countries could be attributed to the difference in variety of potatoes (climatic condition and nutrients effect on the quality of potatoes) especially quantity of reducing sugar and asparagine (Amrein, 2005; Vleeschouwer, 2006). Secondly storage condition of raw material which increases the concentration of reducing sugar in potatoes at below 8°C. Third are the frying condition, difference of oil, temperature and frying time (Amrein, 2005; Eriksson, 2005; Pedreschi, 2006; Vleeschouwer, 2009). Sunflower, cotton and canola oil have polyunsaturated fatty acids which produce acrolein in the course of enzymatic and non-enzymatic maturation cause by lipid oxidation. This is an alternative pathway has been proposed for the formation of acrylamide through acrolein in oil and fat (Eriksson, 2005). On the other hand, acrylamide formation is not significantly influenced by oil oxidation or the presence of any of the hydrolysis products (Mestdagh, 2007). These all conditions help to produce acrylamide in the food product. Therefore, industries should follow the recipes to

Table 1. Acrylamide content (μg/Kg)[a] in branded biscuits (BB), cakes (C) and different varieties of potato chips (PC) samples

Samples	Acrylamide content (μg/Kg)	Samples	Acrylamide content (μg/Kg)
BB-1	113.15 ± 1.85	PC-1	545.0 ± 1.99
BB-2	495.0 ± 3.0	PC-2	65.0 ± 1.00
BB-3	239.5 ± 2.5	PC-3	30.9 ± 1.10
BB-4	113.0± 1.0	PC-4	259.1 ± 2.10
BB-5	507.5 ± 1.5	PC-5	132.0 ± 1.45
BB-6	233.0 ± 3.0	PC-6	57.0 ± 0.85
BB-7	132.0 ± 1.95	PC-7	1323 ± 3.00
BB-8	92.25 ± 1.75	PC-8	47.0 ± 1.17
BB-9	59.0 ± 1.0	PC-9	86.5 ± 0.50
BB-10	286.11 ± 1.88	PC-10	27.1 ± 0.65
BB-11	99.0 ± 1.0	PC-11	243.0 ± 3.00
BB-12	156.1 ± 1.3	PC-12	30.2 ± 1.25
BB-13	140.4 ± 0.6	PC-13	29.0 ± 0.41
BB-14	57.0 ±0.55	PC-14	455.4 ± 1.6
BB-15	52.3 ± 0.70	PC-15	280.0 ±1.50
BB-16	113.0 ± 1.0	PC-16	251.0 ± 2.05
C-1	traces		
C-2	23.5 ± 0.56		
C-3	35.0 ± 0.15		

[a]All values (Average ± Standard deviation of n=3 independent samples) are calculated by excel

reduce acrylamide in products otherwise consumers will be suffering from different diseases.

Industries should be used fresh raw materials for the production of food products to reduce acrylamide level. They should not be used stored potatoes which increase the concentration of reducing sugar at low temperatures and which is in the presence of asparagines may lead to form acrylamide (FAO/WHO 2004). The time of cooking will also effect on the level of acrylamide. It is found that, when the temperature of food rises above 120°C, the rate of acrylamide formation increases quickly with temperature over a limited range (Claus, 2008).

Acrylamide formation in food is pH dependent and optimal pH is 7 for formation of acrylamide in food. The acrylamide formation is inhibited in acidic condition by lowering the pH of the food system. This may attribute to protonating the α-amino group of asparagine, thus can not be engaged in nucleophilic addition reactions with carbonylic source (Zhang, 2007).

Due to health hazard of acrylamide it is necessary to reduce its formation in baked food and frying products is still a major concern in Pakistan. Industries could be produced acrylamide free products by taking the following measures: for potato chips should not be used stored potatoes and if they used stored potatoes then the storage condition should not be below 8°C. Choose potato varieties with low levels of reducing sugars. The fried and baked potato products should be golden yellow and not browned. The frying condition should not be exceed 175°C and oven baking temperature should not be exceed 200°C and drain well of water from the soaked cut potatoes. The baked products such as biscuits and cakes should be avoid excess browning, control the processing time & temperature, formulation and avoid using reducing sugar for products which could increase the level of acrylamide content in baked products. Reducing sugar would be replaced by using flours with a lower asparagines content and sucrose which may reduce the acrylamide content of baked food products.

Conclusion

The standard addition technique has been applied for quantification for the determination of acrylamide in heat processed food samples. In this study, a total of thirty five food samples were collected, sixteen of them were different categories of potato chips, sixteen samples of branded biscuits and cookies and rest three samples were cakes. All of these samples consist of carcinogenic acrylamide contaminant in the range of: 52.3 ± 0.70 to 507.5 ± 1.5 μg/kg (branded biscuits), traces – 35 ± 0.15 μg/kg (cakes) and 27.1 ± 0.65 to 1323.0 ± 3.0 μg/kg (different categories of potato chips). Results demonstrated that acrylamide is commonly found in mentioned food products which are commonly consumed by Pakistani's. These valuable results will help the Pakistani food industries to minimize acrylamide in the products by optimizing processing steps in manufacturing. This will also give awareness to the consumer about the consumable products and they can decide how much quantity can be eaten per day to protect their self from acrylamide contaminant. Acrylamide mitigation and monitoring programmed on acrylamide levels should be initiated in Pakistan by official bodies and industry like other countries in the world.

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