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Determination of the Temperature and Time Required for Formation of Safe Levels of Acrylamide in Bakery Products

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ABSTRACT

Acrylamide as a toxic and carcinogenic substance is produced naturally during high-temperature methods used in baking. **Objectives:** To evaluate the temperature and time required for the formation of safe levels of acrylamide in bakery products. **Methods:** All developed bakery products were evaluated for their chemical elements i.e. fiber, moisture, ash, protein, crude, and crude fat were calculated according to their relevant methods. Bakery product ingredients were procured from the local market of Lahore. Furthermore, developed products were quantified for acrylamide concentration using high-performance liquid chromatography (HPLC) technique. Three samples were prepared Control group (T0), Treatment plan 1 (T1), Treatment plan 2 (T2). From each group, 10g of sample was procured for analysis. **Results:** Pizza treated at T0 (220°C, 15min), T1 (210°C, 20 mints), and T2 (230°C, 10 min) had the following concentration of acrylamide (15.66 ± 3.05, ND, 32.33 ± 2.08 µ/kg), T0 (18°C, 20 mints), T1 (175°C, 25 mints), T2 (195C, 15 mints), showed acrylamide as (66.66 ± 2.51, 42.66 ± 3.05, 90 ± 1.73 µ/kg) and Cake T0 (160-15 mints), T1 (150-20 mints), T2 (170C, 10mins) acrylamide quantified (15.66 ± 2.51, ND, 34.33 ± 2.08µ/kg) and Biscuit (66.66 ± 2.51, 42.66.33 ± 3.05, 90 ± 1.73µ/kg). **Conclusions:** It was concluded that treating T1 (210°C for 20 mints -ND), To (175°C for 25 mints - 48.33 µ/kg), and T3 (150°C for 20 mints-ND) can reduce and mitigate the formation of acrylamide following. Low temperature and high time could serve as an efficient strategy to reduce acrylamide and optimize the process.

INTRODUCTION

Acrylamide is formed during the cooking of plant-based foods when the free amino acid asparagine reacts with reducing sugars such as glucose and fructose in the Maillard reaction [1]. While the Maillard reaction contributes to desirable flavor, colour, and aroma changes in cooked foods, it also produces processing contaminants such as acrylamide [2]. It is known as a processing contaminant. Acrylamide has been classified as potentially carcinogenic to humans by the International Agency for Research on Cancer (IARC) [3]. Acrylamide is a metabolite glycidamide and causes genetic damage when binds with DNA. Various heated products contain acrylamide such as carbohydrate-rich foods [4]. Exposure to high acrylamide levels is said to cause damage to the nervous system. After speedy confirmation of these observations, several activities targeting the origin and extent of exposure of acrylamide in food, its potential risk to human health, and mitigation of acrylamide in foods were initiated [5]. Presently the two methods for the detection of acrylamide are mass spectrometric and non-mass spectrometric-based techniques [6]. The majority of the basic stable industries of food manufacturing focus production of bread and bakery products. The majority of bakery products, bread, and biscuit pizza in the countries that are developed are manufactured through special automated production [7]. Foods that are prepared and eaten in homes are only 60 percent, mostly due to two working parents and individuals who are single parents, have less time to cook

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[8]. Biscuits are considered a famous food product that is consumed by a massive range of populations because of their variety, taste, big shelf life, and comparatively low cost making them economical [9]. Fernandes et al., reported a mass spectrometry method for the detection and the estimation of the quantity of acrylamide in particular food structures of biscuits. The established analytical process revealed 11.8 µg kg−1 and 3.55 as the limit of quantification (LOQ) and limit of detection (LOD). Data analysis was carried out to examine the comparison of the levels of Acrylamide with numerous manufacture specifications, like cooking time and temperature, the color, and water content in various biscuits [10]. A study was designed by Esposito et al., which was carried out to analyze acrylamide in the Italian market targeting bread and sweets. A sample of 200 were assessed and the level of acrylamide formation in bread ranged between 31 to 454 µg/kg and in sweets, it ranged between 204 to 400 µg/kg. No neurotoxic health effect was shown in the data [11]. In a study described by Zilic et al., an acrylamide concentration was detected by baking biscuits at two different time durations at 180 °C for 7, 10, and 13 min. Acrylamide was observed at different baking durations revealing a range of 72.3 - 861.7µg/kg after a baking time of 13 minutes. The data showed no link between the amount of acrylamide in biscuits and free asparagine present in flour. Moreover, rye flour, hulled oat, and durum wheat contain the highest amounts of 859.8,603.2 and 530.3 mg/kg, which produced the highest amount of acrylamide in biscuits after a baking time of 13 minutes. A low amount of acrylamide was observed in biscuits made from refined wheat flour and red maize whole meal flour as they contain a low amount of asparagine as well [12]. A study was investigated by Andacic et al., in 2020. The purpose of this examination was to compare and find out the amount of acrylamide in different baked products. The method used for acrylamide detection was the LC-MS/MS method. The second goal of the study was to assess the average exposure of adults to acrylamide through the bakery food category. A total of 100 samples were assessed, and content ranging below the limit of quantification (LOQ) to 237µg/kg was reported. The dietary bakery food category means exposure of acrylamide estimated as 0.16 µg/kg b.w. per day. For food safety in regards to reducing the level of acrylamide, European regulation has contributed a lot [13].

This study aims to develop acrylamide-free bakery products and to evaluate the quality of acrylamide bakery products

METHODS

The experimental study design was carried out in Food Science and Technology (FST) Lab no.102, University Institute of Food Science and Technology (UIFST), Faculty of Allied Health Sciences (FAHS), The University of Lahore. The duration of research after synopsis approval was 9 months. In this study, oil and other ingredients were procured from the local market of Lahore and then packed in sealed bags and sealed jars to avoid any further contamination until further analysis at the laboratory facility of the University Institute of Food Science and Technology (UIFST) at the University of Lahore. A jury comprised of 10 men and women was selected from The University of Lahore, Lahore. The judges had knowledge of testing terms and they were requested to analyze the multiple samples of cookies, pizza, and cake for taste, crispiness/firmness, color, crust, internal texture, flavor, color, and general acceptability applying a 9-point Hedonicscalewhere9wasequivalent to like extremely and 1 meant dislike extremely [14]. All three developed products (P1, P2, P3) were analyzed by HPLC Acrylamide method. Acrylamide was analyzed by following a method adapted from Gokmen [16]. Bakery products like Pizza, biscuits, and cake were developed. Three samples were prepared Control group (T0) bakery products were baked at a standardized temperature and time, Treatment Plan 1 (T1) was baked at a low temperature with a long time duration, Treatment Plan 2 (T2) was baked at a high temperature and a short duration of time. Bakery products (pizza P1, biscuit P2, and cake P3) were evaluated for their chemical elements i.e. fiber, moisture, ash, protein, crude, and crude fat were calculated according to their relevant methods. Other micronutrients like minerals have been analyzed through various methods. For each separate food type sample (10g) analysis, extraction, and quantification were performed (Table 1).

Table 1: Development of Bakery Products Like Pizza, Biscuits, and Cake

Treatment Plan	P1 Pizza	P2 Biscuits	P3 Cake
T0	220°C for 15 mints	185°C for 20mints	160°C for 15 mints
T1	210°C for 20 mints	175°C for 25mints	150°C for 20 mints
T2	230°C for 10 mints	195°C for 15 mints	170°C for 10 mints

All three developed products were analyzed by the HPLC Acrylamide method. Acrylamide was analyzed by following a method adapted from Gokmen (2005) [15]. Analysis of the food extracts was performed by high-performance liquid chromatography meeting the requirements. Finely, ground (10g) all three samples were prepared. For each separate food type sample (10 g) analysis and extraction were performed. Firstly, all samples were homogenized. 100 ml hexane was added to the sample and was fixed with a flask on a wrist shaker for 20 minutes. Decant hexane and sample were dried on a hot plate. 100 ml acetone and 500 ml distilled water added to the sample and was shaken for 10 minutes. The flask with the sample was placed in a water bath at 40°C for 20 minutes. The sample was placed on a

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hot plate and acetone was evaporated. After the evaporation of acetone 5ml distilled water was added to the residue and filtered from a 0.25-micron syringe filter before the examination. LC-MS assessments were done by an HPLC method containing a binary pump, an auto-sampler, and a thermally controlled column oven, connected to the MS detector furnished with diverse interface employing the following interface variables: drying gas temperature of 350°C, drying gas (N2,20psig) flow of 5L/min, corona current of 5µA, the capillary voltage of 2000V, and nebulizer pressure of 20psig. Onan Atlant is T3 column (150×4.6mm, 3 µm) a critical division was executed by using the isocratic blend of 10 mM formic acid at a flow rate of 0.3 mL/min at 25°C. The LC solvent was administered to the MS arrangement for about 10 to 16 minutes employing MSD software. For the estimation of the quantity of acrylamide in the sample, the observed ions were m/z 72 and 55. Acrylamide was quantified for each sample.

RESULTS

All three products (P1, P2, and P3) were quantified for acrylamide content. ND: (Not-detected)(Table 2).

Table 2: Acrylamide Concentration in Pizza

Pizza	T0	T1	T2
AAµ/kg	15.66 ± 3.05	ND	32.33 ± 2.08

Acrylamide concentration in pizza was analyzed (Figure 2).

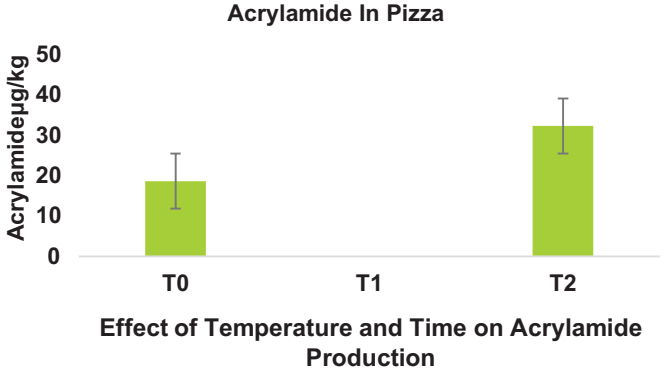

Figure 1: Acrylamide Concentration in Biscuit

Values represent the means of three replicate determinations and Mean + SD values with different superscripts within the same column are significantly different (p≤0.05)(Table 3).

Table 3: Acrylamide Concentration in Biscuit

Biscuit	T0	T1	T2
AAµ/kg	66.66 ± 2.51	42.66.33 ± 3.05	90 ± 1.73

Acrylamide concentration in biscuits was analyzed (Figure 3).

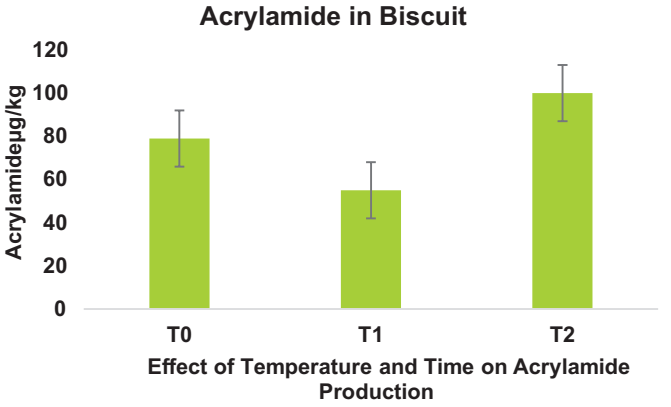

Figure 2: Acrylamide Concentration in Biscuit

Values represent means of three replicate determinations and Mean + SD values with different superscripts within the same column are significantly different (p≤0.05)(Table 4).

Table 3: Acrylamide Concentration in Cake

Cake	T0	T1	T2
AAµ/kg	15.66 ± 2.51	ND	34.33 ± 2.08

Acrylamide concentration in cake was analyzed (Figure 4).

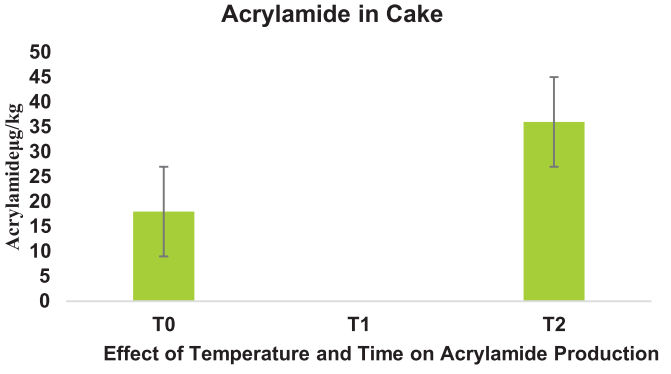

Figure 3: Acrylamide Concentration in Cake

DISCUSSION

An experimental study was conducted to develop and evaluate acrylamide-free selected bakery products. Acrylamide is a toxic substance produced upon heating food products containing reducing sugar and asparagine. Acrylamide does not occur naturally and is formed only during heating treatment, thus known as a processing contaminant. Previously various studies have been conducted to quantify acrylamide amounts in many food products. The study revealed acrylamide T0 (15 µ/kg), and T2 (32.33 µ/kg) whereas in Treatment Plan T1 amount of acrylamide was detected. A study performed by Michalak *et al.*, investigated the amount of acrylamide in pizza and his findings were our result of 33µ/kg [16]. Another study performed previously by Crawford *et al.*, showed the same average value of acrylamide in pizza [17]. The value of acrylamide in T1 was not detectable supported by the findings of Eerola *et al.*, [18]. Detection of acrylamide using

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different strategies helps control measures to stop its formation as it is toxic to human health. In the control group, T0 acrylamide detected was (66.66 µ/kg), T1 (42.66 µ/kg) acrylamide content was slightly low compared to because of low temperature, whereas T2 showed the highest level of acrylamide (90 µ/kg) due to high temperature(°C). Similar findings were performed by Michalak *et al.*, [16]. Another study was similar to our results, performed by Hai *et al.*, [19]. Acrylamide content in control group T0 (15.66µ/kg) was low an amount due to appropriate baking temperature and time, whereas the content of acrylamide was almost double (34.66 µ/kg) as compared to T0 as it was treated at high temperature, moreover in Treatment plan T1, no amount of acrylamide was detected, as it was prepared data low temperature (°C). These concentrations are found in a cake and are within the range of the lowest concentration found in a cake in accordance to a study performed by Hai *et al.*, [19]. Davoodi, *et al.*, reported similar findings by using high-performance liquid chromatography for the detection of acrylamide in cake [20].

CONCLUSIONS

It was conducted that bakery items are consumed globally. The present study targeted three bakery products pizza, biscuit and cake for the evaluation of acrylamide which is a toxic compound formed during processing. The study of baking products at different temperatures and times constitutes an approach to understanding the mechanisms of acrylamide formation during the baking process. Considering the data obtained in our study, treating pizza (210°C for 20 mints -ND), biscuit (175°C for 25 mints - 48.33 µ/kg) and cake (150°C for 20mints-ND) can reduce and mitigate the formation of acrylamide following. Acrylamide formation can be prevented by altering temperature and time. Low temperature and high time could serve as an efficient strategy to reduce acrylamide and optimize the process. Proper attention should be paid to the food processing methods to attain safe food. Food preferences that are alarming to public health must be avoided.

Authors Contribution

Conceptualization: SQ
Methodology: SQ, SB
Formal analysis: AH, MZ, AA
Writing review and editing: RBK, SI

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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