Hindawi
Journal of Chemistry
Volume 2019, Article ID 1425098, 6 pages
https://doi.org/10.1155/2019/1425098

Research Article

Effects of Formulation and Baking Process on Acrylamide Formation in Kolompeh, a Traditional Cookie in Iran

Fatemeh Shakeri,[1] Somayeh Shakeri,[2] Sajad Ghasemi,[3] Antonio Dario Troise,[4] and Alberto Fiore[5]

[1]*Research Center of Tropical and Infection Disease, University of Medical Science, Kerman, Iran*
[2]*Department of Food Science and Engineering, Yazd Branch, Islamic Azad University, Yazd, Iran*
[3]*Department of Food Science, Engineering and Technology, Campus of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*
[4]*Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy*
[5]*Division of Food & Drink School of Science, Engineering and Technology, Abertay University, Dundee DD1 1HG, UK*

Correspondence should be addressed to Alberto Fiore; a.fiore@abertay.ac.uk

Received 26 July 2018; Revised 15 November 2018; Accepted 9 December 2018; Published 2 January 2019

Academic Editor: Susana Casal

Thermal treatments and recipes are two critical aspects for the formation of acrylamide at ordinary household cooking conditions and industrial level. Kolompeh is a traditional Iranian cookie, and the aim of this study was to monitor acrylamide formation in four different recipes: traditional sugary Kolompeh (TSK), traditional simple Kolompeh (TSIK), industrial sugary Kolompeh (ISK), and industrial simple Kolompeh (ISIK). Along with the measurement of reducing sugars, moisture, and pH, acrylamide was quantified by gas chromatography mass spectrometry (GC-MS). Results indicated that acrylamide content was 1758, 1048, 888, and 560 μg/kg for TSK, TSIK, ISK, and ISIK, respectively, revealing that the kind of thermal treatment in combination with higher concentrations of reducing sugars were the major driver for acrylamide formation. In particular, acrylamide concentration in TSIK direct heating was 1.87 times higher than industrial indirect heating treatment, highlighting that domestic preparation of Kolompeh required a specific attention as a source of potential toxic molecule formation.

1. Introduction

Acrylamide (2-propenamide, C_3H_5NO) is usually formed during heat processing of starch-rich foods at temperatures higher than 120°C in presence of asparagine. Roasting, toasting, baking, frying, broiling, and grilling are the typical thermal treatments that promote acrylamide formation due to the presence of an acrylic and amide group. Considering its high reactivity, acrylamide is defined as a potential carcinogenic, reprotoxic, and neurotoxic contaminant for humans [1–3].

Looking at the chemical aspects, asparagine and reducing sugars are the two key precursors of acrylamide formation. In particular, fructose is more reactive than glucose, as a consequence of the lower melting point and the ability to stabilize azomethine ylide, one of the precursors of acrylamide [4, 5]. Polysaccharides as cellulose and pentosans are other efficient precursors of acrylamide while the yield in presence of aldehydes or ketones, such as 2-hydroxy-1-butanal and hydroxy acetone, is higher than the one in presence of glucose [6].

Other minor routes of acrylamide formation include 2-propenal and acrylic acid in presence of amino acids with nitrogen in their side chains such as glutamine, lysine, and arginine or a β-proton in the amino acid adjacent to alanine [4, 7, 8]. The backbone molecules of acrylamide originate from asparagine, which forms the acrylamide by direct deamination and decarboxylation, but the reaction is done inefficiently in the extremely low acid media [8–10].

The Maillard reaction (MR) is the main pathway for acrylamide formation in foods; however, some minor pathways may occur also in non-Maillard environments, as in the case of 3-aminopropionamide, that avoid the requirement of reducing carbonyls [7, 9, 10].

Acrylamide formation is common to a wide variety of food products [11]. Depending on the kind of thermal treatments and the concentration of precursors, acrylamide concentration can go up to 5000 μg/kg. In this frame, bakery products and cookies along with potato products, burgers, nuggets, coffee, and cereals are the most relevant source of acrylamide.

Several methods have been developed for the detection and measurement of acrylamide in food products, where gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS) are the techniques of choice [12]. Recently, rapid methods for detection of acrylamide such as color-indicating methods, enzyme-linked immunosorbent assay (ELISA) methods, supramolecular recognition-based methods, electrochemical biosensing methods, and fluorescent sensing methods have been published [13–15].

Mitigation of acrylamide formation in foods is one of the most challenging aspects related to the Maillard reaction, as the simultaneous formation of desired and undesired molecules are not easily separated [11, 15]. In this respect, control of the reaction mechanisms of acrylamide formation is one of the prerequisites to reduce acrylamide content in foods and the most effective strategies encompass: storage and tuning of the thermal treatment (temperature, time, and water activity), quality of raw materials with the selection of cultivars with low reducing sugar concentration, blanching (temperature and time), reduction of the pH, addition of antioxidants, replacement of reducing sugars, and oxidation of free asparagine into aspartic acid by using asparaginase [16, 17].

Other effective strategies can be designed in the production process such as the use of vacuum baking, radiofrequencies, and ohmic heating. In the case of acrylamide formation, heat treatment of foods needs to be optimized to preserve beneficial outcomes as the formation of flavor, texture, color, and microbiological safety and to limit undesired effects. While for industrial production, this may be achieved more effectively/sustainably by consistent fine-tuning of technological processes, within ordinary household cooking conditions much more attention needs to be dedicated to the recipes and to the thermal exchange, as little modifications can be implemented during production processes [18–20].

The relationship between household cooking conditions and negative outcomes of MR has been investigated by several authors. Trevisan and coworkers demonstrated that home cooking methods did not affect dietary intake of Maillard reaction products (MRPs) in beef and early and furosine as a marker of lysine Amadori compounds predominated in cooked beef [21]. Similarly, Lamberti et al. observed domestic boiling-modified milk protein profile, causing a minor reduction in milk allergenicity [22].

Kolompeh is a traditional cookie of Kerman and Iran and it is composed by two parts: the coating consists of wheat flour, butter, milk, sugar, egg, baking powder ($NaHCO_3$), and bakery yeast, and occasionally spices such as cinnamon, cardamom, and nutmeg are added [23]. The core is characterized by the presence of butter, walnut, almonds, pistachio, and date paste. Along with the common ingredients, Kolompeh cookies can be produced following two different recipes and two different processes. On the one hand, recipes can include reducing sugars in the coating, as in the case of sugary Kolompeh, and on the other hand, sugar is not added to the coating mixture (simple Kolompeh). Along with the addition of sugar, Kolompeh cookies are produced in ordinary household cooking conditions by using direct oven heating and at the industrial level by using indirect heating.

This study deals with the effects of the baking process and formulation of Kolompeh on acrylamide formation, aiming at investigating the correlation of acrylamide, reducing sugars, pH, and moisture content. The final goal is to introduce smart strategies to control acrylamide formation during ordinary household cooking conditions.

2. Materials and Methods

2.1. Materials. Acrylamide and [1,2,3-$^{13}C_3$]-acrylamide (98%) standards purchased from Sigma (St. Louis, MO) and Cambridge Isotope Laboratories, Inc. (Andover, MA), respectively. Methanol, formic acid, *n*-hexane were obtained from Merck (Darmstadt, Germany). Analytical grade sodium thiosulfate pentahydrate, hydrobromic acid, hydrochloric acid, potassium bromide, and bromine (99.8%) were purchased from Sigma (St. Louis, MO).

Food ingredients as wheat flour, sugar, and sunflower oil were purchased from a local market. All chemicals and solvents were of analytical grade.

2.2. Preparation of Kolompeh Cookies. Kolompeh cookies, traditional sugary Kolompeh (TSK), and traditional simple Kolompeh (TSIK) were prepared according to the traditional method, and two different samples were produced according to the following recipe. For TSK samples: 500 g wheat flour and 100 g sugar were added to 300 g margarine and 80 mL cow's milk with 3% fat. Upon careful mixing using a kitchen robot equipped with a hook, 90 g of egg yolks were added along with 2 g salt and 50 mL of distilled water. For TSIK samples, the same recipe was used without the addition of sugars. Twenty biscuits were prepared for each batch and were cooked in triplicate.

The dough of all samples was rolled out to discs with 7 cm diameter and 2 mm thickness using a manual laminator and stored at room temperature. After 30 min, each disc was rounded with 10 g of date paste. Traditional samples (TSK and TSIK) were baked in the traditional oven (direct heating) at 273°C, and industrial samples (ISK and ISIK) were baked in the industrial oven (indirect heating) at 200°C for 15 min.

2.3. Moisture Content and pH. Samples (5 g) were weighed accurately into standardized plates and then dried until constant weight in an oven at 105°C overnight. Moisture content was reported as a percentage according to the standard ISO 6540 : 1980 (International Standard Organisation, 1980). The samples were analyzed in triplicate. For pH determination, finely ground cookies (1 g) were mixed with 100 mL of water and vortexed for 3 min. The mixture was then held at room temperature for 1 hour to separate solid and liquid phases. After carefully removing the supernatant layer, the pH was measured using a CG-837 pH meter (Schott, Mainz, Germany). Analysis was performed in triplicate.

2.4. Reducing Sugar. The nonstoichiometric sugar oxidation process in the presence of alkali (Fehling reagents) was used for quantitative determination of reducing sugars according to the method described by Miller [24]. Each sample was analyzed in triplicate.

2.5. Extraction and Measurement of Acrylamide by Gas Chromatography Mass Spectrometry (GC-MS). GC-MS analysis was performed according to Castel and Wei-Chich [25, 26], using a Varian ion trap Saturn 2200 GC-MS (Agilent Technologies, Santa Clara, CA), and separation of acrylamide and its labeled internal standard was achieved by using a Varian DB5 column (DB5 30 m × 250 μm × 0.25 μm). An aliquot of 10 g sample and 100 μL of d3-acrylamide solution (2 μg·mL^{-1}) were carefully mixed with 10 mL of water. Samples were mixed for 20 minutes and then centrifuged at 4000 rpm at the temperature of 4°C for 10 minutes. The supernatant was filtered using 0.45 μm nylon syringe filters and 300 μL brominating reagent (200 g of potassium bromide, 10 ml of hydrobromic acid, and 160 ml of bromine-saturated water were mixed into a 500 ml volumetric flask and made up to volume with deionised water) added to 3 mL of the filtered sample. The mixture was vortexed and incubated in an ice bath for 1 hour. One drop of 1 N sodium thiosulfate was added to each sample to decompose any remaining bromine. Samples were extracted with 2 mL ethyl acetate and then centrifuged for 10 minutes, and the organic layer was transferred to autosampler vials for analysis. Analysis of 2,3-bromopropionamide (2,3-BPA) and the internal standard was performed in the PCI SIM mode with the following parameters: injector: 180°C; Xfer line: 180°C; trap: 120°C; ionization mode: CI. The SIM masses selected for PCI were 152, 150, 106, and 70 for 2,3-BPA, while for 2- BP(C^{13})A, 154, 152, 110, 108, and 73 were used. The quantification ions were 152 and 154 for 2,3-BPA and 2- BP(C^{13})A, respectively. A calibration curve was prepared in the range 200–2000 μg/kg by following the same brominating procedure described above.

2.6. Statistical Analysis. Acrylamide content in all samples was analyzed for variance (ANOVA) and subjected to Duncan's multiple test by using the SPSS software V. 24. Data are expressed as means of triplicate analyses ± SD. Differences were significant at $p \leq 0.05$. The correlation of reducing sugar concentration and moisture content with acrylamide formation was analyzed by the Pearson correlation coefficient.

3. Results and Discussion

3.1. Effect of Formulation on Acrylamide Formation. Results indicated that acrylamide content in the two different recipes treated with direct and indirect heating, namely, TSK, TSIK, ISK, and ISIK were 1758 ± 4.45, 1048 ± 79.63, 888 ± 12.175, and 560 ± 99.1 μg/kg, respectively (Table 1).

Results of this study showed that TSK and TSIK had a significantly ($p < 0.05$) higher level of acrylamide content than ISK and ISIK (Table 1). The highest level of acrylamide was produced in the TSK sample and the lowest of acrylamide concentration obtained by the ISIK sample. The concentration of reducing sugar also exhibited a clear relationship with acrylamide formation in a complex environment such as Kolompeh cookies; indeed, TSK samples with added sugars showed the maximum level of acrylamide. The dough processing was not controlled in the traditional products that lead to an increase of reducing sugars and cookies baked at elevated temperatures in traditional samples [27]. Increasing reducing sugar concentration and temperature of baking led to an increase of the MR that leads to the increase of acrylamide formation in the traditional sugary Kolompeh. Results reported here were in agreement with other cookies' preparation only for industrial processes with no sugar added, while in presence of sugar and during household production, the concentration was undoubtedly higher than cookies preparation [28–30]. Looking at the concentration of reducing sugars as one of the active precursors of acrylamide formation, results revealed that the concentration in traditional and industrial Kolompeh were 10.04% and 9.84% (Tables 2 and 3). According to our results, the concentration of reducing sugars had a positive Pearson correlation coefficient (0.964, Table 4) with acrylamide formation.

The moisture content in traditional and industrial Kolompeh was 8.11 and 11.19% (Tables 2 and 3). Our investigations indicated the moisture content of Kolompeh showed a negative Pearson correlation (−0.987, Table 4) with acrylamide formation. The moisture content and acrylamide formation, as expected, had an inverse relationship. The moisture content in traditional Kolompeh was lower than industrial Kolompeh, leading to higher acrylamide formation in traditional Kolompeh than industrial products. The evaporation of water promoted the formation of brown compounds and unequivocally increased the reaction rates of MR [31]. High baking temperature and direct heating for traditional samples caused the decrease in moisture content in traditional samples, and the removal of water was one of the key aspects for decarboxylation of Amadori compound and hence for the formation of acrylamide. It was hypothesized that acrylamide formation mainly occurred in the crust independently from the recipe or the thermal treatments. Previous studies reported similar results:

Table 1: Acrylamide content in Kolompehs (μg/kg).

	Min	Max	SD	Mean
TSK	1720	1800	4.45	1758[a]
TSIK	970	1120	79.63	1048[b]
ISK	790	1200	12.175	888[c]
ISIK	480	600	99.1	560[d]

Traditional sugary Kolompeh (TSK), traditional simple Kolompeh (TSIK), industrial sugary Kolompeh (ISK), and industrial simple Kolompeh (ISIK). Significant differences were determined by ANOVA analysis and Duncan test ($p \leq 0.05$). Different letters indicate significant differences.

Table 2: Characters of traditional Kolompeh

	Min	Max	SD	Mean
Moisture content (%)	6.3	10.1	2.0	8.1
Reducing sugar (%)	9.8	10.3	0.07	10.0
pH	5.4	5.9	0.02	5.6

Table 3: Characters of industrial Kolompeh

	Min	Max	SD	Mean
Moisture content (%)	10.4	11.9	0.2	11.2
Reducing sugar (%)	8.5	10.7	0.6	9.8
pH	5.4	5.8	0.01	5.6

Table 4: Correlation coefficient of reducing sugar and moisture content with acrylamide formation.

	Pearson correlation coefficient	p value
Reducing sugar concentration (%)	0.964**	0.002
Moisture content (%)	−0.987**	0.0001

**Correlation significant at the 0.01 level.

moisture, acrylamide, and MR rates were inversely correlated, and the external crust is the principal situ in which the reaction occurs [27, 32].

Our results indicated that the pH did not significantly change (5.60 and 5.63 for the industrial and traditional products). In both industrial and domestic preparation, pH was slightly acid as a consequence of the production of hydrogen atoms during the course of the MR. In this respect, the pH showed a limited impact on the formation of MRPs, in particular acrylamide.

3.2. Effect of Baking Process on Acrylamide Formation. Baking process highly affected acrylamide formation. Direct heating and elevated temperature led to an increase in acrylamide formation, and the hypothesized protection of the coating layer was not effective in mitigating the formation of potentially toxic molecules. Traditional household baking process increased acrylamide concentration from 888 to 1758 μg/kg in the sugary sample, and acrylamide content increased from 560 to 1048 μg/kg in the without sugar sample (Table 1). As previously observed, increasing the temperature of the heat process from 200 to 230°C acrylamide formation increased 2-3 times [33, 34]. Rydberg and coworkers reported similar results for the effect of baking temperature on acrylamide formation, and heating temperature and acrylamide formation exhibited an exponential correlation from 160 to 230°C, while prolonging the thermal treatment over 20 min acrylamide concentration decreased as a consequence of polymerization and Michael addition reaction. [32] Moreover, the presence of free amino groups can influence the elimination of acrylamide as a consequence of the Michael addition [35, 36].

4. Conclusion

The effects of sugar and baking processes on acrylamide formation in Kolompeh biscuits were investigated by taking into account two different recipes, with and without sugar addition, processed in two different conditions that mimicked the industrial scale and household production processes. In both cases, with and without sugars, acrylamide formation was 1.97 and 1.87 times higher in the domestic preparation than industrial process. The optimization of indirect heating at the industrial level guaranteed an effective control of acrylamide formation, while the addition of reducing sugar for the formation of texture or flavor development should be kept under control in order to avoid the formation of potentially toxic compounds. Independently, from the addition of the sugar, household cooking requires particular attention as the direct heating and the higher temperature are effective in the formation of acrylamide. The results of the experiments were as expected, and especially in line with the recent findings of Mesias et al., in order to reduce the exposure to the ACR in the population, a standardization of raw material (limiting the precursors) and increasing the level of automatization will reduce acrylamide [37].

Data Availability

Data are present within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

We gratefully appreciate University of Medical Science, Kerman, Iran. This work has been partially funded by a grant provided by the council for Research at the Research Center of Tropical and Infectious Disease, University of Medical Science, Kerman, Iran. We thank Miss Morag Steele, Abertay University, for the English language revision of manuscript.

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