JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Determination of Acrylamide Levels of “Izmir Gevregi” and Effects of Cooking Parameters on Acrylamide Formation

Esra Alpözen*,† and Ali Üren‡

†Ministry of Food Agriculture and Livestock, İzmir Food Control Laboratory, Bornova İzmir 35100, Turkey

‡Food Engineering Department, Faculty of Engineering and Architecture, Avrasya University, Yomra Trabzon 61250, Turkey

ABSTRACT: Acrylamide level of “Izmir gevregi” samples were detected by liquid chromatography−tandem mass spectrometry. Thirty samples of “Izmir gevregi” supplied from bakeries were analyzed to determine acrylamide levels for the first time. The average acrylamide level of “Izmir gevregi” samples was found to be 68.63 μg/kg. In this study, “Izmir gevregi” samples were cooked in laboratory conditions, first at 3 different time intervals at 280, 285, and 290 °C, after immersing in grape molasses. Shapped “Izmir gevregi” dough samples were also immersed in grape molases, mulberry molasses, and burned sugar solutions at different time intervals and cooked. We detected that acrylamide formation was increased by the incease of cooking temperature, time, and by using the same molasses several times. For the reduction of acrylamide level, KCl was added to dough formulations at different levels. Adding of KCl to dough at 25% and 50% of NaCl content decreased acrylamide values from 101.27 μg/kg to 69.88 and 51.91 μg/kg. By reducing sugar, protein, HMF, pH, and moisture content, L*, a*, and b* parameters were also determined in “Izmir gevregi” samples.

KEYWORDS: *acrylamide, liquid chromatography−tandem mass spectrometry, LC-MS/MS, “Izmir gevregi” samples*

INTRODUCTION

In early 2002, Swedish National Food Administration (SNFA) and University of Stockholm announced that certain foods that are processed or cooked at high temperatures such as during frying, baking, and roasting, especially carbohydrate-rich foods, contain high levels of acrylamide.[1,2] 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.[3,4] The Maillard reaction occurs between amino acids and reducing sugars, proceeding through intermediates that include a Schiff’s base.[5−7]

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 a strong relationship betweeno acrylamide formation and sugar content, especially glucose and fructose.[4,8] Temperature and time of processing, pH, moisture content, reactant concentrations, and type of reactants are important factors of the rate and extent of formation of Maillard reaction products. Decreasing the pH of food is inhibitory to acrylamide formation.[5] Acrylamide can also form to a minor extent from acrolein. Partial hydrolysis of triacylglycerols during frying is followed by dehydration of liberated glycerol to acrolein. Acrolein further oxidizes to acrylic acid, which reacts with ammonia to form acrylamide.[1]

Acrylamide, a neurotoxic compound, was classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans.[9] Acrylamide is mainly reactive through its double bond. In the body, acrylamide can be metabolized to glycidamide, which is a reactive compound formed through epoxidation and considered as a mutagenic and cancer-inducing compound.[1,4,10]

Several analytical methods have been used to quantify acrylamide in foods. 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.[1,11,12]

Cereal-based foods play an important role in the diets of many people in Asia, Africa, the Middle East, and some parts of Europe. “Izmir gevregi” is a traditional fermented cereal product. It is a product of Tatar Turkish who came Izmir from Kırım 450 years ago. There has been no scientific study about the chemical composition of “Izmir gevregi,” but it is a product similar to bread that contains 5.71% protein, 1.07% fat, and 54.7% carbohydrate. During “Izmir gevregi” production, the dough is prepared with flour, water, salt, and yeast. After dough preparation, fermenting, shaping, dipping into hot molasses solution, covering the surface with white sesame seeds, and cooking are realized. After the mixing of flour, water, salt, and yeast, the dough is fermented for 1−2 h. Then shaping in a circle is carried out. Shaped dough is dipped in hot molasses solution for about 10 s. The surface of “Izmir gevregi” is covered with white sesame seeds. Cooking is performed at 280−300 °C for 8−11 min. “Izmir gevregi” is consumed at about 25 °C. “Izmir gevregi” has an important place in poeple’s diets. The Izmir public consumes “Izmir gevregi” every day at breakfast.[13]

Since “Izmir gevregi” is a carbohydrate-rich bakery product, the formation of acrylamide might be expected. Consequently, the aims of the present study were to determine acrylamide levels of “Izmir gevregi” and the effects of cooking parameters on acrylamide formation.

Received: April 16, 2013
Revised: June 30, 2013
Accepted: July 8, 2013
Published: July 8, 2013

MATERIALS AND METHODS

Materials. *Samples.* Thirty "Izmir gevregi" samples were purchased from local bakeries in Izmir (the biggest city in the Aegean region of Turkey). All of the samples were homogenized and stored at −18 °C until analysis for the first part of study.

Reagents. Acrylamide was obtained from Sigma (Steinhein, Germany). 2,2,3-D_3-labeled acrylamide (D_3-AA, isotopic purity >98%) as internal standard was obtained from Polymer Source (Toronto, Canada). Glucose, fructose, potassium hexaciyanoferrate trihydrate, hexane, zinc acetate dihydrate, formic acid, and hydrochloric acid were supplied from Merck (Darmstadt, Germany), methanol was from Sigma (Steinhein, Germany), the HMF standard was from Fluka, 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 were stored in glass containers at −18 °C and contained 250 μg of acrylamide and 250 μg of D_3-AA in 1 mL, separately. Then 0.4 mL of acrylamide and 0.4 mL of D_3-AA standard stock solutions were diluted to 100 mL with HPLC grade distilled water just before use to prepare working standard solutions of 1 mg/L. Carrez I solution was prepared by dissolving 10.6 g of potassium hexaciyanoferrate trihydrate in 100 mL of water and carrez II solution by dissolving 24 g of zinc acetate dihydrate in 100 mL of water.

Preparation and Cooking of "Izmir Gevregi". Flour, water, salt, and yeast were mixed at certain levels. The dough was fermented for 90 min. At the end of the fermantation time, the dough was divided into pieces of 90 g. Then shaping into a circle was carried out. Shaped dough was dipped into hot molasses solution for about 10 s at 75 °C. The surface of "Izmir gevregi" was covered with white sesame seeds. As acrylamide was formed in the crust, the radius of samples was controlled in all stages during the making of "Izmir gevregi". Cooking was performed at certain temperature and time intervals. The effect of different cooking parameters on acrylamide formation was studied.

Effect of Temperature and Time. For determining the effect of temperature and time on acrylamide formation, "Izmir gevregi" samples were cooked at 280 °C at 8.5; 10; 11.5 min, 285 °C at 8; 9.5; 11 min, 290 °C at 7.5; 9; 10.5 min.

Effect of Boiling Time and Molasses Type. For determining the effect of boiling time and molassses type on acrylamide formation, "Izmir gevregi" samples were cooked at 290 °C for 9 min. Grape molasses, mulberry molasses, and burnt sugar solution were used. "Izmir gevregi" is mostly made by bakeries using grape molasses solution. Additionally, these solutions are used several times because of economic reasons. Therefore, these solutions were boiled 4 h after dipping "Izmir gevregi" samples three times in this study. Eventually, 4 groups of samples were obtained for each solution.

Effect of the Addition of KCl to Dough Formulation. KCl has not been used in "Izmir gevregi" so far. In this study, 25% KCl was added to dough, 50% of the NaCl amount, instead of NaCl.

Methods. *Sample Preparation for LC-MS/MS Analysis.* Acrylamide anaylsis was performed according to Alpözen et al.[14] In this method, 6 g of a previously homogenized "Izmir gevregi" sample was weighed into a beaker. Following the addition of 33 mL of distilled water, the mixture was mixed for 25 min. Then, 1.5 mL of Carrez I and 1.5 mL of Carrez II solutions were added. The sample was mixed for a further 5 min and then centrifuged at 16770*g* for 15 min. The precipitate was discarded, and the supernatant was extracted with 20 mL of hexane to remove apolar matrix components. Twenty milliliters of aqueous solution was taken, and 2 mL of 1 mg/L D_3-AA solution was added. The pH of the solution was adjusted to 2.3 by 6 M HCl solution and then diluted to 25 mL with distilled water. A Waters Oasis MCX SPE cartridge (New York, USA) was used for further cleanup. The pass through strategy for the SPE cleanup was applied to retain the matrix interferences. The Oasis MCX cartridge was conditioned consecutively with 1 mL of methanol and 1 mL of water at a rate of one drop per second. Air was passed through the cartridge by pushing the injector to remove the remaining water. One milliliter 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. The extract was filtered through a 0.45 μm nylon filter prior to analysis. Analysis was performed in duplicate.

LC-MS/MS Analysis. Chromatographic separations were performed by using an Agilent 1200 liquid chromatograph (Santa Clara, USA) connected to a 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. The optimized MS instrument parameters were drying gas temperature (N_2, 350 °C) with a flow rate of 12 L/min, nebulizer pressure of 40 psi, capillary voltage of 4 kV, fragmenter voltage of 60 eV for D_3-AA and 70 eV for acrylamide, and collision energy of 10 eV for each transition. Ions monitored were 55 for the quantification ion of acrylamide and 58 for the quantification ion of D3-acrylamide in the samples.

Mobile phase was prepared by mixing solvent A and solvent B in a ratio of 90:10 (v/v). 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 × 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.

Determinations of Protein, Reducing Sugar, HMF, Moisture, pH, and Color. *Determination of Protein Content.* Protein analysis was performed according to the AOAC 990.03.2000 method[15] using a Leco FP-528 apparatus (Michigan, USA). This instrument works by the Dumas principle, gives the nitrogen content (w/w) of food material, and converts this value to protein content through multiplying by 5.7, which is the suitable coefficient for bakery products. Analysis was performed in duplicate.

Determination of Reducing Sugar Content. Reducing sugar contents (glucose + fructose) were determined according to the IHC (International Honey Commission) 2002 method with some modifications.[16] Fructose and glucose were extracted with water, centrifuged, filtered through 0.45 μm PTFE filters, and injected to Agilent 1100 HPLC-RID (Santa Clara, USA). Analysis was performed by a Zorbax (NH_2) column (250 mm × 4.6 mm, 5 μm, Santa Clara, USA). The mobile phase was prepared from acetonitrile and distilled water (85:15) (v/v). Column temperature was 30 °C, and the flow rate of the mobile phase was 1 mL/min with an injection volume of 20 μL. Analysis was performed in duplicate.

Determination of HMF Content. HMF contents were determined according to the IHC (International Honey Commission) 2002 method with some modifications.[16] HMF was extracted with water, centrifuged, filtered through 0.45 μm PTFE filters, and injected into Agilent 1100 HPLC-RID (Santa Clara, USA). Analysis was performed using a C_{18} column (250 mm × 4.6 mm, 5 μm, Santa Clara, USA). The mobile phase was prepared from distilled water and methanol (90:10) (v/v). Column temperature was 30 °C, and the flow rate of the mobile phase was 1 mL/min with an injection volume of 20 μL. Analysis was performed in duplicate.

Determination of pH. The pH values of "Izmir gevregi" samples were measured with a Sartorius digital pH meter (Goettingen, Germany) in triplicate.[17]

Determination of Moisture Content. Moisture contents of "Izmir gevregi" samples were measured according to TS 1135 ISO 712 by using a Nüve Drying Oven (Ankara, Turkey) in triplicate.[18]

Determination of Color Parameters. L*, a*, and b* CIE color parameters of "Izmir gevregi" samples were measured by Hunterlab (Model Colorflex, Virginia, USA) as four replicates.

Statistical Analysis. Statistical analyses were realized with the SPSS 16.0 statistics package program. The statistical analyses of data were achieved by using one-way analysis of variance (ANOVA), Duncan's post-test, and Pearson's correlation test.

RESULTS AND DISCUSSION

Linearity was observed up to 750 μg/kg "Izmir gevregi" with a correlation coefficient of 0.999. Recovery rate, limit of detection (LOD), and limit of quantification (LOQ) values of the method

Table 1. Acrylamide, Protein, Reducing Sugar, HMF and Moisture Contents, pH, and Color Parameters of "Izmir Gevregi" Samples[a]

									color parameters		
sample no.	acrylamide (μg/kg)	protein (%, w/w)	fructose (mg/kg)	glucose (mg/kg)	reducing sugar (mg/kg)	HMF (mg/kg)	% moisture (w/w)	pH	L*	a*	b*
1	96.73 c ± 0.2	9.28 lm ± 0.03	8482 f ± 1	4304 c ± 1	12786 ç ± 1	30.51 i ± 0.02	23.81 l ± 0.04	5.897 hi ± 0.006	61.78 j ± 0.03	9.67 q ± 0.03	29.06 mn ± 0.04
2	76.43 j ± 0.17	10.94 d ± 0.03	5757 s ± 1	2419 r ± 1	8176 q ± 1	41.11 ç ± 0.03	22.78 o ± 0.09	5.87 j ± 0.01	54.56 w ± 0.008	11.05 d ± 0.03	28.72 p ± 0.05
3	87.89 e ± 0.24	10.31 ghi ± 0.03	6172 p ± 1	2704 m ± 1	8876 o ± 1	16.76 r ± 0.05	25.16 j ± 0.17	5.90 hi ± 0.006	65.05 b ± 0.03	8.74 t ± 0.03	29.02 n ± 0.04
4	42.02 z ± 0.17	10.46 fgh ± 0.08	10091 c ± 1	4007 ç ± 1	14098 c ± 1	33.52 g ± 0.06	25.76 i ± 0.04	5.96 f ± 0	65.08 b ± 0.03	8.19 u ± 0.02	27.99 t ± 0.03
5	49.72 u ± 0.10	10.37 fghi ± 0.07	8030 ğ ± 1	3173 h ± 1	11203 h ± 1	49.78 a ± 0.03	25.03 j ± 0.14	6.00 c ± 0.1	58.14 u ± 0.02	10.34 i ± 0.03	29.49 k ± 0.02
6	79.85 g ± 0.25	10.41 fghi ± 0.04	5851 q ± 1	2136 s ± 1	7987 r ± 1	5.78 v ± 0.03	23.51 m ± 0.20	5.98 d ± 0	59.63 q ± 0.02	10.83 f ± 0.03	31.03 f ± 0.02
7	42.43 y ± 0.05	10.51 f ± 0.09	7135 k ± 1	2908 k ± 1	10043 j ± 1	25.53 l ± 0.07	23.06 n ± 0.20	5.853 k ± 0.006	58.7 r ± 0.02	11.22 c ± 0.02	31.01 f ± 0.04
8	69.69 p ± 0.16	10.25 ij ± 0.17	7994 h ± 1	3451 ğ ± 1	11445 ğ ± 1	22.37 n ± 0.05	25.59 i ± 0.24	6.0 c ± 0	62.73 e ± 0.03	9.42 r ± 0.02	29.11 m ± 0.03
9	48.96 x ± 0.19	10.01 k ± 0.12	8311 g ± 1	3756 e ± 1	12067 g ± 1	34.14 f ± 0.03	23.27 mn ± 0.20	5.9 h ± 0	60.62 m ± 0.03	10.47 h ± 0.03	29.97 h ± 0.05
10	76.86 i ± 0.27	8.71 o ± 0.04	20945 b ± 1	13174 b ± 1	34119 b ± 1	31.53 h ± 0.01	22.05 p ± 0.12	5.757 n ± 0.006	61.48 k ± 0.04	10.95 e ± 0.03	31.21 e ± 0.05
11	49.09 x ± 0.14	10.46 fgh ± 0.06	6435 n ± 1	3051 j ± 1	9486 m ± 1	21.98 o ± 0.04	27.62 d ± 0.10	5.5 q ± 0.01	60.24 o ± 0.03	10.26 j ± 0.04	29.82 i ± 0.06
12	49.49 uv ± 0.11	11.00 cd ± 0.05	4265 w ± 1	2616 n ± 1	6881 t ± 1	30.33 j ± 0.02	27.21 e ± 0.09	5.783 m ± 0.006	58.27 t ± 0.02	10.52 g ± 0.03	29.38 l ± 0.05
13	120.21 a ± 0.26	9.42 l ± 0.02	7735 i ± 1	2748 l ± 1	10483 i ± 1	19.17 p ± 0.02	20.2 q ± 0.04	6.007 c ± 0.006	51.12 z ± 0.02	11.93 a ± 0.02	32.34 a ± 0.02
14	59.13 p ± 0.07	9.14 mn ± 0.03	6323 o ± 1	2474 o ± 1	8797 p ± 1	3.76 y ± 0.02	28.97 a ± 0.07	6.843 a ± 0.006	66.64 a ± 0.03	7.68 v ± 0.05	26.82 z ± 0.05
15	76.82 i ± 0.18	10.51 f ± 0.01	8774 d ± 1	3623 g ± 1	12397 f ± 1	38.53 d ± 0.03	23.76 l ± 0.14	5.92 g ± 0	60.22 o ± 0.04	10.18 k ± 0.06	28.88 o ± 0.07
16	54.33 t ± 0.26	10.5 fg ± 0.06	5109 ş ± 1	2424 p ± 1	7533 s ± 1	10.8 t ± 0.03	27.85 cd ± 0.04	5.473 r ± 0.006	59.99 p ± 0.03	10.01 mn ± 0.04	29.61 j ± 0.07
17	80.97 f ± 0.32	8.99 n ± 0.002	6602 m ± 1	3123 i ± 1	9725 k ± 1	30.53 i ± 0.02	24.08 k ± 0.17	5.923 g ± 0.006	63.54 c ± 0.05	10.03 m ± 0.03	28.3 s ± 0.02
18	80.15 g ± 0.27	11.7 a ± 0.03	4342 v ± 1	1267 z ± 1	5609 w ± 1	26.85 k ± 0.03	23.39 m ± 0.10	5.86 i ± 0,01	56.28 v ± 0.04	9.89 o ± 0.01	27.09 y ± 0.04
19	74.57 k ± 0.16	11.13 c ± 0.07	8894 ç ± 1	3815 d ± 1	12709 d ± 1	45.37 b ± 0.04	26.02 h ± 0.05	6.02 b ± 0	60.45 n ± 0.04	10.03 mn ± 0.02	29.96 h ± 0.04
20	54.71 s ± 0.17	10.41 fghi ± 0.05	3786 x ± 1	1813 t ± 1	5599 x ± 1	13.59 ş ± 0.04	25.81 hi ± 0.06	5.89 i ± 0	60.92 l ± 0.09	9.46 r ± 0.10	28.43 r ± 0.07
21	119.33 b ± 0.25	9.41 l ± 0.01	32026 a ± 1	25197 a ± 1	57223 a ± 1	15.85 s ± 0.03	22.67 o ± 0.09	5.8 l ± 0	51.23 y ± 0.02	11.76 b ± 0.02	32.08 b ± 0.02
22	54.51st ±0.10	10.28 hi ± 0.02	4498 ü ± 1	1600 ü ± 1	6098 v ± 1	25.6 l ± 0.03	24.27 k ± 0.21	5.9 h ± 0	58.6 s ± 0.02	10.51 gh ± 0.02	29.05 mn ± 0.02
23	49.1 x ± 0.08	11.03 cd ± 0.03	4783 u ± 1	1714 u ± 1	6497 u ± 1	32.26 ğ ± 0.03	28.05 c ± 0.17	6.0 c ± 0	63.35 d ± 0.07	8.97 s ± 0.03	28.61 q ± 0.06
24	92.52 d ± 0.17	10.45 fgh ± 0.02	8704 e ± 1	3730 f ± 1	12434 e ± 1	43.79 c ± 0.03	26.91 f ± 0.10	5.753 n ± 0.006	62.37 f ± 0.01	9.79 p ± 0.01	31.44 d ± 0.01
25	62.72 n ± 0.24	10.48 fg ± 0.06	3257 y ± 1	1486 w ± 1	4743 y ± 1	38.06 e ± 0.03	22.8 o ± 0.25	5.707 o ± 0.006	62.17 h ± 0.01	10.13 l ± 0.01	29.48 k ± 0.03
26	58.35 r ± 0.22	10.76 e ± 0.15	2299 z ± 1	1416 x ± 1	3715 z ± 1	6.9 u ± 0.03	23.14 n ± 0.16	5.63 p ± 0	52.36 x ± 0.03	10.22 jk ± 0.02	31.55 c ± 0.04
27	49.17 vx ± 0.04	10.09 jk ± 0.04	5823 r ± 1	1592 u ± 1	7415 ş ± 1	18.37 q ± 0.03	28.36 b ± 0.05	5.97 e ± 0	61.91 i ± 0.04	9.42 r ± 0.02	27.73 v ± 0.06
28	59.93 o ± 0.17	10.42 fghi ± 0.02	6964 l ± 1	1927 ş ± 1	8891 n ± 1	23.43 m ± 0.04	26.27 g ± 0.15	5.9 h ± 0	62.28 g ± 0.04	10 n ± 0.03	29.8 i ± 0.04
29	64.73 m ± 0.17	11.38 b ± 0.05	4912 t ± 1	1335 y ± 1	6247 ü ± 1	5.62 w ± 0.03	26.33 g ± 0.20	6.02 b ± 0	60.44 n ± 0.03	10.78 f ± 0.04	30.62 g ± 0.02
30	78.57 h ± 0.22	10.25 ij ± 0.15	7160 j ± 1	2422 q ± 1	9582 l ± 1	2.34 z ± 0.02	26.71 f ± 0.17	5.983 d ± 0.006	63.36 d ± 0.06	10.8 f ± 0.02	27.87 u ± 0.02

[a]The numbers in parentheses are standard deviations. Different matching letters in a column mean significant differences according to Duncan's test ($P < 0.05$).

were estimated on "Izmir gevregi" samples. Recovery rate was found to be 99.3% with LOD and LOQ values of 1.5 μg/kg and 5.0 μg/kg, respectively. Certified reference materials of crisp bread with T-3021 and T-3026 reference codes supplied by FAPAS (UK) were analyzed. Acrylamide contents of these samples were found in the range cited in the certificates.

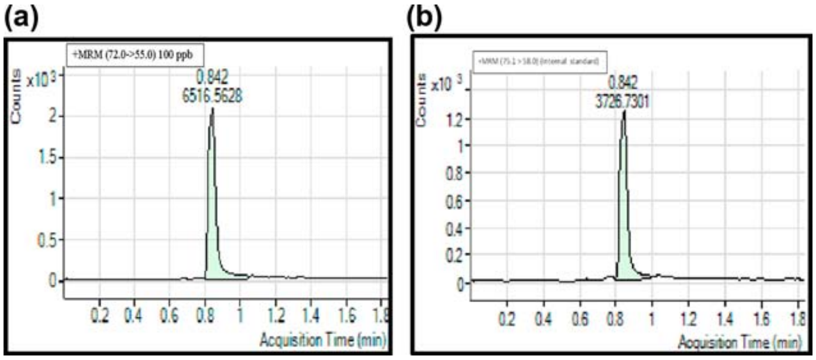

Figure 1. LC-MS/MS chromatogram of acrylamide fragment ion m/z 55.0 (a) in standard acrylamide solution and (b) in "Izmir gevregi" sample.

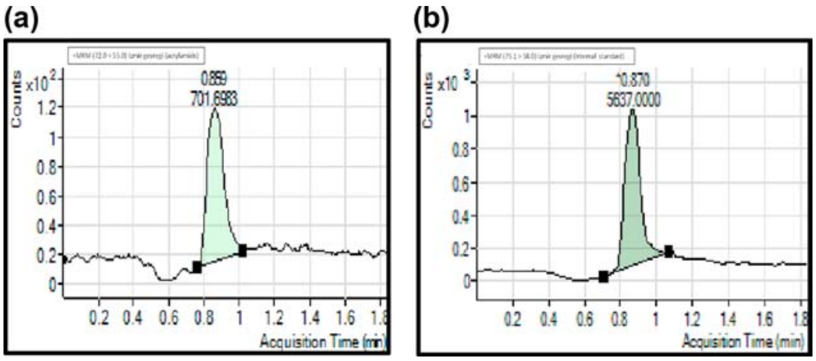

Figure 2. LC-MS/MS chromatogram of D_3-acrylamide fragment ion m/z 58.0 (a) in standard D_3-acrylamide solution (b) in the "Izmir gevregi" sample.

Acrylamide, Protein, Reducing Sugars, HMF, Moisture, pH, and Color Values of "Izmir Gevregi" Samples under Survey. Concentrations of acrylamide in 30 "Izmir gevregi" samples from different manufacturers are shown in Table 1. As is seen in the table, acrylamide contents of "Izmir gevregi" samples varied from 42.02 μg/kg to 120.21 μg/kg with an average content of 68.63 μg/kg. Significant differences were detected among acrylamide contents of "Izmir gevregi" samples ($p < 0.05$). In Table 1, different matching letters in a column indicate significant differences. The chromatograms of the fragment ion m/z 55.0 for acrylamide in standard solution and the "Izmir gevregi" sample are demonstrated in Figure 1 and for the fragment ion 58.0 internal standard are demonstrated in Figure 2.

Protein, reducing sugars, moisture, pH, and color values of "Izmir gevregi" samples were determined and tabulated in Table 1. The protein contents of "Izmir gevregi" samples varied from 8.71% to 11.70% (w/w) with a mean value of 10.30%. Significant differences were detected among protein contents of "Izmir gevregi" samples ($p < 0.05$). According to Pearson's correlation test, a negative correlation was obtained between acrylamide contents and protein contents of "Izmir gevregi" samples ($p < 0.05$, $r = -0.367$). The glucose contents of samples were determined between 1267 mg/kg and 251967 mg/kg, and the mean value was 3713 mg/kg. Significant differences were demonstrated among glucose contents of "Izmir gevregi" samples ($p < 0.05$). According to Pearson's correlation test, a positive correlation was obtained between acrylamide contents and glucose contents of "Izmir gevregi" samples ($p < 0.01$, $r = 0.485$). Corresponding values for fructose were from 2299 mg/kg to 32026 mg/kg with a mean value of 7715 mg/kg. Significant differences were also detected among fructose contents of "Izmir gevregi" samples ($p < 0.05$). According to Pearson's correlation test, a positive correlation was obtained between acrylamide contents and fructose contents of "Izmir gevregi" samples ($p < 0.01$, $r = 0.492$). Total reducing sugar contents of "Izmir gevregi" samples varied from 3715 mg/kg to 57223 mg/kg, and the mean value was 11429 mg/kg. Significant differences were detected among total reducing sugar contents of "Izmir gevregi" samples ($p < 0.05$). According to Pearson's correlation test, a positive correlation was obtained between acrylamide contents and total reducing sugar contents of "Izmir gevregi" samples ($p < 0.01$, $r = 0.491$).

HMF contents of "Izmir gevregi" samples varied from 2.34 mg/kg to 49.78 mg/kg, and the mean value was 24.81 mg/kg. Significant differences were established among HMF contents of "Izmir gevregi" samples ($p < 0.05$). No statistically significant correlations were obtained between acrylamide contents and HMF values of "Izmir gevregi"samples ($p < 0.01$, $p < 0.05$).

The moisture contents of "Izmir gevregi" samples varied from 20.2% to 28.97% (w/w), and the mean value was 25.01%. Significant differences were established among moisture contents of "Izmir gevregi" samples ($p < 0.05$). According to Pearson's correlation test, a negative correlation was obtained between acrylamide and moisture contents of "Izmir gevregi" samples ($p < 0{,}01$, $r = -0{,}499$). This finding was in accordance with the results of Sadd et al.[19] in bakery products and Viklund et al.[20] in fried potatoes. The pH of "Izmir gevregi" samples varied from 5.47 to 6.84, and the mean value of pH was 5.90. There were significant differences among pH values of "Izmir gevregi" samples ($p < 0.05$). No statistically significant correlations were obtained between acrylamide contents and pH values of "Izmir gevregi" samples ($p < 0.01$, $p < 0.05$).

L* values of "Izmir gevregi" samples varied from 51.12 to 66.64, and the mean value of L* was 60.12. Significant differences were detected among L* values of "Izmir gevregi" samples ($p < 0.05$). According to Pearson's correlation test, a negative

Table 2. Acrylamide, Protein, Reducing Sugar, HMF and Moisture Contents, pH, and Color Parameters of "Izmir Gevregi" Samples Cooked at Different Temperatures and Times[a]

sample no.	acrylamide (μg/kg)	% protein (w/w)	fructose (mg/kg)	glucose (mg/kg)	total reducing sugar (mg/kg)	HMF (mg/kg)	moisture (w/w)	pH	color parameters L*	a*	b*
1	81.73 i ± 0.27	10.73 c ± 0,11	2581 c ± 1	2898 a± 1	5479 b ± 1	3.94 i ± 0.03	26.97 ab± 0.53	5.81 d ± 0.01	57.50 b ± 0.05	11.28 i ± 0.04	30.70 d ± 0.04
2	91.58 f ± 0.21	11.17 c ± 0.08	1752 g ± 1	2313 c ± 3	4065 f ± 3	8.49 f ± 0.02	24.55 d ± 0.35	5.83 c ± 0.01	52.28 e ± 0.08	12.48 e ± 0.09	30.97 c ± 0.24
3	103.23 c ± 0.21	11.64 a ± 0.07	1513 h ± 1	1092 h ± 1	2605 h ± 1	16.44 c ± 0.01	21.87 f ± 0.24	5.84 c ± 0.01	46.39 g ± 0.02	13.09 c ± 0.06	29.74 f ± 0.09
4	84.27 h ± 0.26	10.74 c ± 0.16	3321 a ± 1	2614 b ± 3	5935 a ± 3	5.40 h ± 0.02	27.38 a ± 0.15	5.88 a ± 0	58.66 a ± 0.03	11.66 h ± 0.01	31.98 a ± 0.06
5	94.93 e ± 0.23	11.01 b ± 0.04	2509 d ± 1	2130 e ± 2	4639 d ± 2	9.57 e ± 0.01	26.52 b ± 0.34	5.87 a ± 0	52.60 d ± 0.01	12.01 f ± 0.03	30.06 e ± 0.07
6	109.62 b ± 0.19	11.57 a ± 0.09	2079 f ± 1	1717 g ± 2	3796 g ± 2	26.07 b ± 0.02	22.24 ef ± 0.24	5.85 b ± 0.01	43.59 h ± 0.03	13.43 b ± 0.04	29.00 h ± 0.06
7	88.55 g ± 0.22	10.69 c ± 0.04	2783 b ± 2	2156 d ± 3	4939 c ± 3	6.76 g ± 0.04	27.10 ab ± 0.22	5.85 b ± 0.01	56.66 c ± 0.01	11.84 g ± 0.06	31.44 b ± 0.03
8	101.47 d ± 0.19	11.11 b ± 0.09	2457 e ± 2	1927 f ± 2	4384 e ± 2	10.93 d ± 0.03	25.21 c ± 0.05	5.85 b ± 0.01	48.25 f ± 0.03	12.67 d ± 0.03	29.23 g ± 0.10
9	115.55 a ± 0.09	11.47 a ± 0.09	1468 i ± 2	1078 i ± 1	2546 i ± 2	28.08 a ± 0.03	22.65 e ± 0.48	5.85 b ± 0.01	43.31 i ± 0.03	13.60 a ± 0.05	29.07 h ± 0.07

[a]The numbers in parentheses are standard deviations. Different matching letters in a column mean significant differences according to Duncan's test ($P < 0.05$).

correlation was obtained between acrylamide contents and L* values of "Izmir gevregi" samples ($p < 0.05$, $r = -0.372$). a* values of "Izmir gevregi" samples varied from7.68 to 11.93 with a mean value of 10.11. Significant differences were detected among a* values ($p < 0.05$). According to the correlation test, a positive correlation was obtained between acrylamide contents and a* values of "Izmir gevregi" samples ($p < 0.05$, $r = 0.388$). b* values of "Izmir gevregi" samples varied from 26.82 to 32.34, and the mean value of b* was 29.52. Significant differences were also observed among b* values of "Izmir gevregi" samples ($p < 0.05$). According to Pearson's correlation test, a positive correlation was obtained between acrylamide contents and b* values of "Izmir gevregi" samples ($p < 0.05$, $r = 0.381$). Correlations between acrylamide contents and color parameters (L*, a*, and b*) were in agreement with the results of Viklund et al.[20]

Acrylamide, Protein, Reducing Sugars, HMF, Moisture, pH, and Color Values of "Izmir Gevregi" Samples in Evaluating the Temperature and Time Effect. "Izmir gevregi" samples were cooked at 280 °C at 8.5; 10; 11.5 min, 285 °C at 8; 9.5; 11 min, 290 °C at 7.5; 9; and 10.5 min. Acrylamide, protein, reducing sugars, HMF, moisture, pH, and color values of "Izmir gevregi" samples were determined and tabulated in Table 2. The effects of temperature and time on acrylamide content is shown in Figure 3. Significant differences were detected among all parameters (acrylamide, protein, reducing sugars, HMF, moisture, pH, and color value contents) of "Izmir gevregi" samples ($p < 0.05$). In Table 2, different matching letters in a column indicate significant differences. Significant differences were detected among temperature groups only for pH values, but for others (acrylamide, protein, reducing sugars, HMF, moisture and color value contents), no significant differences were detected ($p < 0.05$).

According to Pearson's correlation test, a positive correlation was obtained between acrylamide contents and protein contents ($p < 0.01$, $r = 0.873$); between acrylamide and HMF ($p < 0.05$, $r = 0.952$); between acrylamide contents and a* values ($p < 0.05$, $r = 0.966$); a negative correlation was obtained between acrylamide and glucose ($p < 0.01$, $r = -0.900$); between acrylamide and fructose ($p < 0.01$, $r = -0.723$); between acrylamide and reducing sugar ($p < 0.01$, $r = -0.865$); between acrylamide and moisture ($p < 0.01$, $r = -0.860$); between acrylamide and L* values ($p < 0.05$, $r = -0.975$); between acrylamide contents and b* values ($p < 0.05$, $r = -0.879$). No statistically significant correlations were obtained between acrylamide contents and pH values of "Izmir gevregi" samples ($p < 0.01$, $P < 0.05$). Negative correlations between acrylamide and fructose, and glucose and reducing sugar show that sugar was used up during cooking in the Maillard reaction. Therefore, the formation of acrylamide was increased, sugar content was decreased as beginning fructose, glucose, and reducing sugar contents of "Izmir gevregi" doughs are the same. Negative correlation between acrylamide and L* values show that while acrylamide was increased, color was darkened, and therefore, the L* values are decreased.

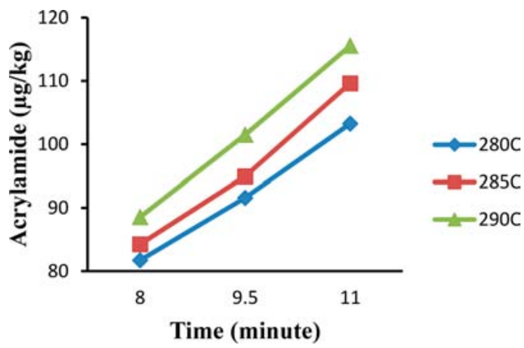

Figure 3. Effects of time and temperature on acrylamide content of "Izmir gevregi" samples.

Acrylamide, Protein, Reducing Sugars, HMF, Moisture, pH, and Color Values of "Izmir Gevregi" Samples in Evaluating the Effect of Boiling Different Types of Molasses. "Izmir gevregi" samples were cooked at 290 °C for 9 min by using grape molasses, mulberry molasses, and burnt sugar solutions. Acrylamide, protein, reducing sugars, HMF, moisture, pH, and color values of "Izmir gevregi" samples were determined and tabulated in Table 3. Significant differences were

Table 3. Acrylamide, Protein, Reducing Sugar, HMF and Moisture Contents, pH, and Color Parameters of "Izmir Gevregi" Samples Cooked after Dipping in Boiled Molasses Solutions[a]

sample no.	acrylamide (μg/kg)	% protein (w/w)	fructose (mg/kg)	glucose (mg/kg)	total reducing sugar (mg/kg)	HMF (mg/kg)	% moisture (w/w)	pH	color parameters L*	a*	b*
1	98.93 g ± 0.15	11.35 bc ± 0.02	2376 c ± 1	1602 e ± 1	3978 b ± 1	11.04 e ± 4.09	25.08 c ± 0.37	5.86 c ± 0.01	53.88 f ± 0.01	11.83 f ± 0.02	30.29 ef ± 0.05
2	106.33 f ± 0.18	11.19 bcd ± 0.13	2440 a ± 15	1906 a ± 1	4346 a ± 15	11.59 cde ± 0.18	24.91 c ± 0.19	5.89 ab ± 0.01	54.13 e ± 0.02	12.03 d ± 0.03	30.41 d ± 0.07
3	112.85 d ± 0.21	11.43 b ± 0.13	2292 d ± 2	1446 g ± 1	3738 d ± 2	12.12 abcde ± 2.49	24.47 de ± 0.13	5.86 c ± 0.01	50.73 l ± 0.01	11.54 h ± 0.01	28.73 j ± 0.08
4	119.27 b ± 0.20	11.28 bc ± 0.13	1351 j ± 2	1312 h ± 2	2663 h ± 2	13.16 de ± 4.47	25.91 b ± 0.02	5.85 cd ± 0.01	51.95 i ± 0.03	12.33 c ± 0.03	30.71 c ± 0.05
5	96.94 h ± 0.19	11.38 bc ± 0.06	2422 b ± 2	1557 f ± 2	3979 b ± 2	10.62 cde ± 0.61	25.53 b ± 0.20	5.90 a ± 0.01	51.19 k ± 0.01	11.88 e ± 0.07	28.90 i ± 0.08
6	107.13 e ± 0.30	11.10 bcd ± 0.13	1970 f ± 3	1674 d ± 2	3644 e ± 3	10.97 ab ± 8.72	25.83 b ± 0.13	5.89 ab± 0.01	56.28 b ± 0.02	11.11 j ± 0.03	29.31 h ± 0.01
7	113.98 c ± 0.25	11.03 bcd ± 0.01	1813 g ± 1	1253 k ± 2	3066 g ± 2	11.39 de ± 2.67	26.41 a ± 0.16	5.85 cd ± 0.01	56.03 c ± 0	10.90 k ± 0.01	29.38 h ± 0.04
8	120.60 a± 0.18	11.28 bc ± 0.11	677 k ± 2	1063 l ± 2	1740 j ± 2	15.24 bcde ± 2.49	24.98 c ± 0.06	5.84 cd ± 0.12	51.83 j ± 0.02	12.69 b ± 0.03	30.84 b ± 0.09
9	72.20 l ± 0.10	10.80 d ± 0.22	2135 e ± 1	1683 c ± 3	3818 c ± 3	15.83 a± 7.07	24.71 c ± 0.15	5.86 c ± 0.10	55.99 d ± 0.02	11.52 h ± 0,03	30.22 f ± 0.04
10	76.55 k ± 0.24	11.92 a ± 0.49	1658 h ± 1	1792 b ± 3	3450 f ± 3	16.08 abcd ± 0.03	24.34 de ± 0.21	5.88 b ± 0.10	53.38 h ± 0.03	11.72 g ± 0,03	29.76 g ± 0.07
11	81.27 j ± 0.23	10.96 cd ± 0.13	1373 i ± 3	1269 j ± 2	2642 i ± 3	16.22 abcd ± 0.05	25.84 b ± 0.37	5.81 e ± 0.10	57.90 a ± 0.04	11.22 i ± 0.03	30.32 e ± 0.01
12	87.00 i ± 0.19	11.18 bcd ± 0.09	1383 i ± 2	1278 i ± 1	2661 h ± 2	17.30 abc ± 0.02	24.12 e ± 0.40	5.84 d ± 0.10	53.74 g ± 0.02	12.77 a ± 0.03	31.66 a ± 0.06

[a]The numbers in parentheses are standard deviations. Different matching letters in a column mean significant differences according to Duncan test ($P < 0.05$).

Table 4. Acrylamide, Protein, Reducing Sugar, HMF and Moisture Contents, pH, and Color Parameters of "Izmir Gevregi" Samples Cooked after Adding KCl to Its Dough[a]

	acrylamide (μg/kg)	% protein (w/w)	fructose (mg/kg)	glucose (mg/kg)	total reducing sugar (mg/kg)	HMF (mg/kg)	% moisture (w/w)	pH	color parameters L*	a*	b*
standard Izmir gevregi	101.27 c ± 1.18	11.23 a ± 0.03	2474 c ± 3	2216 c ± 3	4690 c ± 4	9.52 a ± 0.03	25.16 c ± 0.10	5.93 a ± 0.01	53.41 c ± 0.03	12.03 a ± 0.03	29.48 a ± 0.06
Izmir gevregi cooked after adding 25% KCl of NaCl amount	69.88 b ± 1.86	11.34 a ± 0.20	2874 b ± 1	2780 b ± 3	5654 b ± 3	4.39 c ± 0.03	25.86 a ± 0.32	5.87 c ± 0.01	54.17 b ± 0.02	10.70 c ± 0.03	27.36 c ± 0.04
Izmir gevregi cooked after adding 50% KCl of NaCl amount	51.91 a ± 1.54	11.01 a ± 0.25	3285 a ± 1	3124 a ± 3	6414 a ± 3	6.39 b ± 0.02	25.47 b ± 0.11	5.90 b ± 0.01	54.53 a ± 0.02	10.98 b ± 0.02	28.01 b ± 0.05

[a]The numbers in parentheses are standard deviations. Different matching letters in a column mean significant differences according to Duncan's test ($P < 0.05$).

detected among all parameters (acrylamide, protein, reducing sugars, HMF, moisture, pH, and color value contents) of "Izmir gevregi" samples ($p < 0.05$). In Table 3, different matching letters in a column indicate significant differences. Significant differences were detected among molasses groups only for acrylamide, but for others (acrylamide, protein, reducing sugars, HMF, moisture, and color value contents), no significant differences were detected ($p < 0.05$). Results show that while boiling time was increased or the same solution was used several times or for days, acrylamide formation was increased.

Acrylamide, Protein, Reducing Sugars, HMF, Moisture, pH, and Color Values of "Izmir Gevregi" Samples in Evaluating the Effect of the Addition of KCl to Dough Formulation. KCl has not been used in "Izmir gevregi" so far. In this study, KCl was added to the dough formulation at different levels, and then acrylamide analysis was performed. The effect of KCl on reduction of acrylamide formation was evaluated. KCl was added to the dough at 25% and 50%. Acrylamide values decreased by 69.88 and 51.91 ppb.

Acrylamide, protein, reducing sugars, HMF, moisture, pH, and color values of "Izmir gevregi" samples were determined and tabulated in Table 4. Significant differences were not detected among protein values but were detected among other parameters (acrylamide, glucose, fructose, reducing sugars, HMF, moisture, pH, and color value contents) of "Izmir gevregi" samples ($p < 0.05$). In Table 4, different matching letters in a column indicate significant differences.

A rapid, accurate, precise, low cost, and nontoxic LC-MS/MS method was used for trace determination of acrylamide in "Izmir gevregi" samples. Acrylamide contents of "Izmir gevregi" samples were determined for the first time in this study. By making samples in the laboratory, effects of cooking parameters on acrylamide formation were determined. It was detected that acrylamide formation was increased by inceasing cooking temperature, by cooking time, and by dipping in the same molasses solutions. However, acrylamide formation was decreased by adding KCl to dough formulation. KCl may be used instead of NaCl for decreasing acrylamide formation and for decreasing NaCl consumption.

AUTHOR INFORMATION

Corresponding Author

*Tel: +90 232 4351481. Fax: +90 232 4624197. E-mail: esra_alpozen@hotmail.com.

Funding

We thank Turkish Ministry of Food, Agriculture and Livestock (Project No: TAGEM/GY/10/03/01/165) for financial support.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Claeys, W. L.; De Vleeschouwer, K.; Hendrickx, M. E. Quantifying the formation of carcinogens during food processing:Acrylamide. *Trends Food Sci. Technol.* **2005**, *16*, 181−193.

(2) Zhang, Y.; Zhang, G.; Zhang, Y. Occurrence and analytical methods of acrylamide in heat-treated foods review and recent developments. *J. Chromatogr., A* **2005**, *1075*, 1−21.

(3) Amrein, T. M.; Andres, L.; Escher, F.; Amado, R. Occurrence of acrylamide in selected foods and mitigation options. *Food Addit. Contam.* **2007**, *1* (24), 13−25.

(4) Barutçu, I.; Şahin, S.; Sumnu, G. Acrylamide formation in different batter formulations during microwave frying. *LWT - Food Sci. Technol.* **2009**, *42*, 17−22.

(5) Shaikh, M. B.; Tarade, K. M.; Bharadwaj, V. R.; Annapure, U. S.; Singhal, R. S. Effect of an alkaline salt (papad khar) and its substitute (2:1 sodium carbonate:sodium bicarbonate) on acrylamide formation in papads. *Food Chem.* **2009**, *113*, 1165−1168.

(6) Yaylayan, V.; Wnorowski, A.; Locas, C. Why asparagin needs carbohydrates to generate acrylamide. *J. Agric. Food Chem.* **2003**, *51*, 1753−1757.

(7) Zhang, Y.; Zhang, Y. Formation and reduction of acrylamide in maillard reaction: A Review based on the current state of knowledge. *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 521−542.

(8) Pedreschi, F.; Kaack, K.; Granby, K. Acrylamide content and color development in fried potato strips. *Food Res. Int.* **2006**, *39*, 40−46.

(9) IARC. Acrylamide. In Some Industrial/Chemicals; *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; International Agency for Research on Cancer: Lyon, France, 1994; Vol. *60*, pp 389−433.

(10) Mucci, L.; Dickman, P.; Steineck, G.; Adami, H.; Augustsson, K. Dietary Acrylamide and cancer of the large bowel, kidney and bladder: Absence of an association in a population-based study in Sweden. *Br. J. Cancer* **2003**, *88*, 84−89.

(11) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Analysis of Acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998−5006.

(12) Wenzl, T.; Beatriz de la Calle, M.; Anklam, E. Analytical methods for the determination of acrylamide in food products; a review. *Food Addit. Contam.* **2003**, *20* (10), 885−902.

(13) Alpözen, E.; Güven, G.; Üren, A. Three Traditional Bakery Products of Izmir: "Izmir gevregi", Kumru and Boyoz. II; Traditional Food Semposium: 2009; 27−29 May, 2009.

(14) Alpozen, E.; Guven, G.; Ozdestan, O.; Uren, A. Determination of acrylamide in three different bread types by an in-house validated LC-MS/MS method. *Acta Aliment.***2013**, DOI: 10.1556/AAlim.2013.3333.

(15) AOAC 990.03.2000, Determination of protein content, USA, 2000.

(16) IHC (International Honey Commission), Minutes of the IHC meetings, Switzerland, 2002.

(17) TS12230, Milföy Hamuru; Ankara: Institute of Turkish Standards, 2010.

(18) TS 1135 ISO 712, Tahıl ve Tahıl Ürünleri- Rutubet Muhtevası Tayini Rutin Referans Metot Ankara Institute of Turkish Standards, 2001.

(19) Sadd, P. A.; Hamlet, C. G.; Liang, L. Effectiveness of methods for reducing acrylamide in bakery products. *J. Agric. Food Chem.* **2008**, *56*, 6154−6161.

(20) Viklund, G. A.; Olsson, K. M.; Sjöholm, I. M.; Skog, K. I. Acrylamide in crisps: Effect of blanching studied on long-term stored potato clones. *J. Food Compos. Anal.* **2010**, *23*, 194−198.