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Research Paper / Araştırma Makalesi

Effect of Cooking Method on Heterocyclic Aromatic Amines Contents of Sucuk

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ABSTRACT

In this study, we aimed to find the influence of different levels of doneness (rare, medium, over-cooked) on the formation of heterocyclic aromatic amines (HCAs) in Turkish fermented beef sausages (sucuk) cooked by pan-frying method. Six types of HCAs were determined at three different temperatures. Determination of HCA was made using a high performance liquid chromatography (HPLC). IQ, MeIQx and 4,8-DiMeIQx were detected and quantified with DAD detector and PhIP, norharman and harman were determined with fluorescence detector. External standard and recovery methods were both used for the calculation amount of HCAs to obtain results that are more accurate. HCA and soluble protein analyses were performed in rare, medium and over-cooked sucuk samples. Moisture, ash, protein, lipid contents and pH analyses were performed in raw sucuk samples. Total HCA content of sucuk samples was found between 0.65 and 17.90 ng/g. Total HCA content of over-cooked sucuk samples were higher than rare-cooked sucuk samples ($P<0.05$). Norharman was found as the most abundant HCA in sucuk samples, followed by PhIP, 4,8-DiMeIQx and IQ, respectively.

Keywords: Heterocyclic aromatic amine, HPLC, Sucuk

Sucuk Örneklerinin Heterosiklik Aromatik Amin İçeriğine Pişme Yönteminin Etkisi

ÖZ

Bu çalışmada farklı pişirme derecelerinin (az pişmiş, normal pişmiş ve çok pişmiş) tavada kızartma yöntemi ile pişirilen sucuk örneklerinin heterosiklik aromatik amin (HCA) içerikleri üzerine olan etkilerinin belirlenmesi amaçlanmıştır. Örneklerde üç farklı sıcaklıkta, altı farklı HCA belirlenmiştir. HCA'ların analizi yüksek performanslı sıvı kromatografisi (HPLC) cihazı ile gerçekleştirilmiştir. IQ, MeIQx ve 4,8-DiMeIQx DAD dedektörde, PhIP, norharman ve harman floresans dedektörde tespit edilmiştir. Doğru sonuçlar elde etmek için HCA miktarlarının hesaplanmasında dış standart ve geri kazanım yöntemleri kullanılmıştır. Tüm pişmiş sucuk örneklerinde HCA ve çözünür protein analizleri gerçekleştirilmiştir. Pişmemiş sucuk örneklerinde nem, kül, protein, lipid ve pH analizleri gerçekleştirilmiştir. Sucuk örneklerinin toplam HCA içeriği 0.65 ile 17.90 ng/g arasında bulunmuştur. Çok pişmiş sucuk örneklerinin toplam HCA içeriği, az pişmiş örneklerinkinden fazla bulunmuştur ($P<0.05$). Örneklerde en fazla bulunan HCA norharmandır. Norharmandan sonra sucuk örneklerinde sırasıyla PhIP, 4,8-DiMeIQx ve IQ tespit edilmiştir.

Anahtar Kelimeler: Heterosiklik aromatik amin, HPLC, Sucuk

INTRODUCTION

Heterocyclic aromatic amines (HCAs) occur while thermal processing of protein-rich foods [1]. These substances have been reported in thermally processed muscle tissues [2]. Currently above 25 different types of HCAs were known in cooked proteinaceous foods such as meat [3]. The most common HCAs are the thermally formed ones [4]. For instance, in beef, chicken and fish, HCAs can be found in higher concentrations, whereas HCA concentrations were lower in sausages and pork have upon cooking [5].

The amount of HCAs depends on the meat type, duration of cooking, cooking temperature, and browning degree in the course of heating [6]. It was also reported that heat and mass transfer, fat content, lipid oxidation, antioxidants, carbohydrates, free amino acids, and creatine, pH and water activity can affect the formation of HCAs [7-9]. To decrease the level of HCAs in meat products, antioxidants (vitamin E) or antioxidant-rich herbs (sage or thyme) can be added [10]. Type of cooking; such as grilling, frying, barbecuing, broiling or roasting; is another parameter that effects the formation and concentration of HCAs in meat [11].

HCAs have mutagenic and carcinogenic characteristics. For instance, HCAs can be 100-times and 2000-times more mutagenic compared to aflatoxin B1 and benzo[a]pyrene, respectively [7]. Rohrmann et al. [12] reported that probability of colorectal cancer increased upon concentration of HCAs more than 41.4 ng per day. In another study, it was also mentioned that consumed amount of food and intake frequency of HCAs were found important, besides the type of food and cooking method. As these compounds have mutagenic, carcinogenic or other health related adverse effects, the intake by human should be decreased as much as possible [9].

Currently, solid phase and solid-liquid extractions are used to extract HCAs and HPLC equipped with UV or fluorescence detectors is used to determine the HCAs. In addition, electro-spray ionization tandem mass spectrometry (LC/ESI-MS/MS) was found to detect HCAs in meat samples with a high accuracy and solid phase extraction by usage of two different cartridges was reported as the best sample preparation technic. Identification of these compounds using high technology equipment with an increased sensitivity could give rise to detect novel HCAs that have not been discovered yet [13].

Fermented meat products gain increasing attention worldwide and particularly in Turkey, sucuk as a fermented sausage is the most commonly consumed type [14]. Sucuk is prepared mainly by mixing lamb and beef meat with tail fat. Antimicrobials, antioxidants, starter culture and some spices are also added under controlled atmosphere to this mixture. When the mix, which is called sucuk dough, is ready, it is stuffed into an artificial or natural casing, that allows water evaporate and then fermentation occurs at a specified temperature and time [14]. According to Turkish food legislation, sucuk contains 16% protein (as minumum) and the ratio of moisture content to protein content should be below 2.5. The ratio of fat content to protein content should be below 2.5. pH values of sucuk samples are acceptable as 5.4 as maximum value [15].

Because the formation of carcinogens is largely be affected by cooking methods and temperatures, recommendations about limiting their production during cooking could be the best public health protection system. Sucuk is one of the popular traditional Turkish meat products. To our knowledge, there is only one study [16] about the determination of HCA content of sucuk in literature. These authors studied the influence of spice and lipid types on HCAs in barbecued sucuk. However, they gave no data about norharman and harman content of sucuk samples. Therefore, the objective of this study was to determine the amounts of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-f] quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP), 9H-pyrido[3,4-b]indole (norharman) and 1-methyl-9H-pyrido[3,4-b]indole (harman) in ten fermented beef sausage (sucuk) samples bought from the markets in Turkey. And also, we aimed to investigate the effect of doneness (pan frying) levels on these heterocyclic aromatic amines in sucuk samples for the first time in literature. Our study is the first study about making survey about HCAs content of pan-fried sucuk samples. Pan-frying is the most common way for consumption of sucuk samples. So this study's results give information about prediction of HCAs exposure of people by consumption sucuk.

MATERIALS and METHODS

Materials

Samples

Ten fermented beef sucuk samples of the different brand names were analyzed for the determination of HCA content. Three samples having different production number from each brand were used for the determination of HCA content. Sucuk samples were bought from the markets in Izmir, Turkey at the beginning of their shelf life. The width of sucuk sample was adjusted as 5 cm. Sucuk samples were cooked in a pan with an electrical cooker. These samples were cooked at 180°C for 90 seconds (rare cooked), 180 seconds (medium cooked) and 300 seconds (over cooked), respectively. So, for each sucuk sample, three different levels of doneness (rare, medium and over cooked) were obtained.

Reagents

HPLC or analytical-reagent grade chemicals were used in all analyses. The Human Corporation Zeener Power I water purification system (Seoul, Republic of Korea) was used for obtaining ultra-pure distilled water.

The most abundant six HCAs found in cooked meat products were determined in this study. IQ, MeIQx, 4,8-DiMeIQx, PhIP, and harman bought from Toronto Research Chemicals (Toronto, Canada). Norharman was purchased from Sigma (Steinheim, Germany). Two different cartridges (An Oasis MCX cartridge 3 cm^3/60 mg, Waters, Milford, Massachusetts, USA and (Accubond C_{18} cartridge, 3 cm^3/200 mg, Agilent Technologies, Santa Clara, CA, USA) were used for sample preparation. Zinc sulfate heptahydrate was obtained from Riedel-de Haen (Seeize, Germany). HPLC-grade methanol and acetonitrile, acetic acid, Coomassie Brillant Blue G-250, phosphoric acid (85%), sodium chloride, potassium dihydrogen phosphate, potassium hydrogen phosphate, ethylenediaminetetraacetic acid (EDTA), bovine serum albumin, chloroform, and hydrochloric acid (37%) were all obtained from Merck (Darmstadt, Germany).

One hundred mg/L stock individual HCA solutions in methanol were prepared. Standard solutions were prepared for HCA analyses for plotting calibration curve.

Methods

Heterocyclic Aromatic Amines Content Analysis

The method described by Özdestan et al. [17] was used for sample preparation for HCA analysis. Quantification of IQ, MeIQx and 4,8-DiMeIQx were made by HPLC equipped with a diode array detector (DAD) (254 nm for IQ; 263 nm for MeIQx, and 4,8-DiMeIQx). BDS Hypersil C_{18} (5 µm particle size, 150 mm × 4.6 mm i.d., Thermo Scientific) column was used for separation of HCAs. Chromatographic separations were performed by HPLC using a fluorescence detector (340 nm and 420 nm as excitation and emission wavelengths) for quantification of PhIP, norharman and harman. Injection volumes, mobil phases and flow rates were shown in Table 1. Two different gradient programs were used for separation of HCAs given in Table 1 (a) and (b).

Table 1 (a). Gradient elution program for separation of IQ, MeIQx, and 4,8 DiMeIQx

Time (minute)	A[1](%)	B (%)
0	100	0
12	100	0
20	70	30
29	70	30
34	0	100
35	0	100
44	0	100
49	100	0
55	100	0

[1]A: methanol/acetonitrile/water/acetic acid (8:14:76:2, v/v/v/v) at pH 5.0, B: acetonitrile, Injection volume:20 µL

Table 1 (b).Gradient elution program for separation of PhIP, harman, and norharman

Time (minute)	A[1] (%)	B (%)	Flow rate (mL/min)
0	90	10	0.5
10	75	25	0.5
15	0	100	0.5
20	0	100	0.5
22	90	10	0.5
27	90	10	0.5

[1]A: 0.5 M ammonium acetate B:methanol Injection volume:15 µL

Soluble Protein Analysis

Bradford [18]'s method was used for the determination of soluble protein content. The concentration of soluble protein in sucuk sample was determined by plotting calibration curve prepared by albumin fraction V standard.

Proximate Composition Analysis

Moisture contents of samples were measured in accordance with Anon. [19]. AOAC [20] method was used for determination of ash content. Crude fat content was determined using the methanol-chloroform extraction method according to Folch et al. [21]. Crude protein content was analyzed in accordance with the Dumas method.

pH Determination

For sample preparation, 10 g of sample was homogenized in 100 mL distilled water. A pH meter (Hanna HI-221) was used for the determination pH value of samples. pH meter was calibrated at 20℃ with buffer solutions [22].

Apparatus

An Agilent 1200 liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) was used for quantification of HCAs. Determination of soluble protein content was performed by a UV–visible spectrophotometer (Cary 50, Varian, UK). pH of samples were determined with a pH meter (Hanna HI-221, USA). Crude protein content of samples were determined by LECO FP-528 (Saint Joseph, USA). Nüve ES500 (Ankara, Turkey) oven was used for the determination of moisture content. IKA T25 Ultra-Turrax homogenizer (25,000 rpm, Staufen, Germany) and IEC CL31-CL31R Multispeed centrifuge (Thermo, San Diego, CA) were used in the extraction of samples.

Statistical Analysis

SPSS 24.0 statistics package program was used for evaluation of results statistically. One-way analysis of variance (ANOVA), Duncan post-test and Pearson correlation test were used (P<0.05). All trials were made as 3 replicates.

RESULTS and DISCUSSION

Moisture, ash, crude fat, crude protein contents and pH values of raw sucuk samples were given in Table 2. Similar results for chemical and physic-chemical analysis of raw sucuk samples were obtained with the previous studies [16, 23]. And these values were generally suitable according to Turkish Food Legislation [15]. pH values of raw sucuk samples were found in the range of 4.87 and 5.57 with the mean value of 5.23. Moisture content of raw sucuk samples were between 33.10% and 52.50% with the mean value of 41.12%. Ash content of raw sucuk samples were found between 2.24% and 3.48% with the mean value of 2.88%. Crude fat and protein content of samples were between 22.73% and 44.56% and between 15.17% and 21.17%, respectively. Mean crude fat and protein content of samples were found as 36.31% and 17.79%, respectively.

Table 2. Moisture, ash, crude fat, crude protein contents (%) and pH values of sucuk samples (Mean ± standard deviation)

Samples	pH	Moisture content (%, w/w)	Ash content (%, w/w)	Crude fat content (%, w/w)	Crude protein content (%, w/w)
S1	5.41^{b}±0.00	33.10^{g}±0.15	3.48^{a}±0.04	44.56^{a}±0.44	17.44^{c} ±0.13
S2	5.29^{c}±0.03	39.31^{e}±0.84	3.22^{b}±0.00	33.39^{f}±0.11	21.17^{a} ±0.33
S3	5.47^{a}±0.01	52.50^{a}±0.56	2.92^{c} ±0.00	22.73^{h}±0.02	17.54^{c} ±0.58
S4	5.01^{e}±0.01	42.87^{c} ±0.58	2.27^{f}±0.03	37.66^{d}±0.35	17.71^{c} ±0.41
S5	5.30^{c}±0.01	35.82^{f}±0.96	3.28^{b}±0.06	41.83^{b}±0.20	16.44^{d}±0.54
S6	5.11^{d} ±0.01	39.19^{e}±0.52	2.24^{f}±0.01	35.57^{e}±0.35	15.17^{e} ±0.01
S7	5.45^{ab}±0.01	38.56^{e}±0.13	3.24^{b}±0.01	38.50^{c}±0.67	19.80^{b} ±0.25
S8	5.01^{e} ±0.04	40.99^{d} ±0.21	2.65^{e}±0.026	34.88^{e}±0.18	16.54^{d} ±0.54
S9	4.87^{f}±0.03	48.28^{b}±0.39	2.69^{e} ±0.04	32.13^{g}±0.00	15.98^{de}±0.11
S10	5.44^{ab} ±0.04	40.55^{d}±0.59	2.85^{d} ±0.02	41.89^{b}±0.01	20.15^{b}±0.35

[a]Different matching letters in the same column mean significant differences between the samples according to Duncan test (P < 0.05), All the analyses were performed triplicate

The average recoveries for IQ, MeIQx, 4,8-DiMeIQx, harman, norharman and PhIP were found between 68.9 and 87.8%. Limit of detection (LOD) and limit of quantification (LOQ) values for this method were found as 0.27 and 0.80 ng/g for IQ, 0.86 and 2.59 ng/g for MeIQx, 1.40 and 4.40 ng/g for 4,8-DiMeIQx, 0.04 and 0.13 ng/g for PhIP, 0.65 and 1.96 ng/g for harman, 0.26 and 0.79 ng/g for norharman, respectively [17].

In Table 3, HCA amounts of sucuk samples prepared at different temperatures were shown. External standard and recovery methods were both used for the calculation amount of HCAs to obtain results that are more accurate.

IQ was only detected in one of the analyzed cooked sucuk sample (S3). IQ content of rare, medium and over-cooked sucuk samples were determined as 1.77 ng/g, 0.84 ng/g and 3.84 ng/g, respectively. These values were similar with the literature values. But no IQ was detected in nine out of ten samples. This could be related with the matrix and chemical composition of sucuk. According to Skog et al. [6], IQ was not found in a solution containing creatine, glucose and amino acids that was cooked until 150-225°C between 0-120 minutes. On the other hand, Balogh et al. [24] reported that IQ was found as 0.7 and 1.3 ng/g in meatball samples cooked for 12 and 20 minutes at 175℃. In another study, IQ contents of hamburger meatball cooked at 170, 200 and 225°C were found as 1.1 ng/g, 5.46 ng/g and not determined, respectively [25]. Öz and Kaya [8] determined IQ content of grilled meatball at 175°C and 200°C and found 1.40 ng/g and 5.46 ng/g, respectively. Unal et al. [16] found IQ contents of barbecued sucuk samples between 0.85 ng/g and 5.96 ng/g.

Table 3. IQ, 4,8-DiMeIOx, PhIP and norharman contents of cooked sucuk samples (ng/g) (Mean ± standard deviation)

	IQ			4,8-DiMeIQx			PhIP			Norharman			Total HCA		
S	Rare cooked	Medium cooked	Over cooked	Rare cooked	Medium cooked	Over cooked	Rare cooked	Medium cooked	Over cooked	Rare cooked	Medium cooked	Over cooked	Rare cooked	Medium cooked	Over cooked
S1	nd	Nd	nd	nd	nd	nd	nd	nd	nd	9.01 ±0.20	nd	10.44±0.11	9.74^{b} ±0.61	1.52^{g}±0.34	12.23^{b} ±1.15
S2	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	9.62 ±0.30	nd	nd	4.56^{d} ±0.27
S3	1.77±0.23	0.84 ±0.40	3.84 ±0.59	3.57±0.56	5.98 ±0.35	2.91 ±0.76	nd	nd	nd	9.44 ±0.02	9.02 ±3.02	10.02±0.00	14.84^{a} ±0.78	16.10^{a} ±0.69	17.89^{a}±0.46
S4	nd	Nd	nd	nd	nd	nd	nd	9.75±0.98	nd	nd	nd	nd	nd	9.75^{c}±0.98	1.83^{g} ±0.05
S5	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.41^{d} ±0,01	3.197^{f} ±0.167	3.63^{e} ±0.03
S6	nd	Nd	nd	nd	nd	nd	nd	nd	nd	6.21 ±0.31	6.01 ±0.30	nd	6.21^{c} ±0.30	6.02^{e} ±0.26	nd
S7	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	6.11 ±0.51	10.54±1.41	0.65^{f} ±0.01	7.47^{d} ±0.44	11.47^{c}±0.90
S8	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S9	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.08^{e} ±0.13	nd	2.63^{f} ±0.07
S10	nd	Nd	nd	nd	nd	nd	nd	6.02 ±0.71	8.53 ±0.28	nd	9.31 ±0.30	6.01 ±1.01	nd	12.52^{b} ±1.73	17.71^{a}±0.57

[a] Different matching letters in the same column mean significant differences between the samples according to Duncan test (P < 0.05)
nd=not detected, All the analyses were performed triplicate

MeIQx was not detected in any of the analyzed samples in accordance with literature [6]. MeIQx was not quantified in cooked meatball samples for 8 minutes at 165–200°C by Johansson et al. [26]. Busquets et al. [27] did not found MeIQx in hamburger meatball samples cooked for 11.2 minutes at 175–200°C. MeIQx content of barbecued sucuk samples were found concentrations up to 0.21 ng/g by Unal et al. [16].

4,8-DiMeIQx was detected in only one of the analyzed cooked sucuk sample (S3). 4,8-DiMeIQx content of rare, medium and over-cooked sucuk samples were determined as 3.57 ng/g, 5.98 ng/g and 2.91 ng/g, respectively. These values were similar with the previous values reported in literature [28]. But no 4,8-DiMeIQx was detected in nine of the ten samples. This could be related with the matrix and chemical composition of sucuk. And also, the occurrence of HCAs is connected with meat type, pH, temperature and water activity. Heat and mass transfer, lipids, lipid oxidation, antioxidants and cooking methods such as barbecuing and grilling have some effects on the accumulation of HCAs. Natural or synthetic antioxidants are commonly used to prevent HCA formation in foods. Sucuk contains spices having antioxidant activity, this could be the main reason for detecting any 4,8-DiMeIQx in samples. Lipid content was also an important factor for the formation of HCAs. The influence of lipids on the formation of HCAs could be clarified by the enhanced formation of pyridines, pyrazines and Strecker aldehydes in the Maillard reaction or by the production of free radicals by means of thermic oxidation, or both.

Oz and Kaya [8] cooked meatballs containing black pepper with electrical grill. According to their results, 4,8-DiMeIQx was not determined in any samples. But they found different amounts of 4,8-DiMeIQx in control samples. This shows us that the spices are very important components affecting HCA amount of samples so it is normal not to detect 4,8-DiMeIQx in sample like sucuk that contains different spices. Unal et al. [16] found no 4,8-DiMeIQx in seven of the analyzed twelve sucuk samples. They quantified 4,8-DiMeIQx between 0.08 ng/g and 0.19 ng/g in five of the analyzed twelve samples.

PhIP was found in only two samples. PhIP concentration was found between 6.02 ng/g and 9.75 ng/g in accordance with literature [29]. Oz and Kaya [8] cooked meatballs containing black pepper with electrical grill. They did not found PhIP at any samples. PhIP was not found in five of the analyzed twelve samples by Unal et al. [16]. The concentration of PhIP was reported in between 0.36 ng/g and 1.94 ng/g.

Harman could not be detected in any of the cooked sucuk samples. Ahn and Grün [30] was found 10.88 ng/g harman in cooked meatball samples at 200°C for 20 minutes. However, these cooking temperatures are very high compared to our cooking temperatures. Therefore, the reason of formation or measurable higher concentration of harman could be the degree of heating temperature and time.

The concentration of norharman was found between 6.21 and 9.44 ng/g for rare-cooked; 6.01 and 9.31 ng/g for medium-cooked; and 6.01 and 10.54 ng/g for over-cooked sucuk samples. However, the concentration of norharman could not be detected in four samples. These values were similar compared to the literature values [30]. Ahn and Grün [30] determined norharman content of control cooked beef meatball samples as 5.55 ng/g, 0.5% grape seed extract containing samples as 5.38 ng/g and 1% grape seed extract containing samples as 5.16 ng/g.

Total HCA content of rare, medium and over-cooked samples were found between 0.65 ng/g and 14.84 ng/g; 1.52 ng/g and 16.10 ng/g; 1.83 ng/g and 17.89 ng/g, respectively. Mean total HCA content of rare, medium and over-cooked samples were calculated as 3.69 ng/g, 5.66 ng/g and 6.77 ng/g, respectively. It has been known that possibility of colorectal cancer increases upon consumption of HCAs more than 41.4 ng per day [12]. According to this information, it is recommended to consume pan-fried sucuk less than 2.3 g/day. Total HCA contents of rare, medium and over-cooked samples were significantly different from each other ($P<0.05$). The highest total HCA content was belong to over-cooked sucuk samples and the lowest total HCA content was belong to rare cooked sucuk samples. According to Pearson correlation test, no significant correlation was found between total HCA content and lipid, protein, ash, dry matter, soluble protein content of rare, medium and over-cooked sucuk samples ($P<0.05$). No correlation was obtained between total HCA content and pH values of sucuk samples except over-cooked samples ($P<0.05$).

In a previous study, beef and chicken meatballs containing a 0.5% (w/w) pomegranate (PSE) and grape seed extract (GSE) were cooked using different methods and IQ, MeIQx, 4,8-DiMeIQx, PhIP, norharman, and harman were detected [31, 32]. In the control, GSE added and PSE added beef meatballs, total HCA contents were reported as 154.63 ng/g, 182.65 ng/g and 83.23 ng/g, respectively.

Unal et al. [16] evaluated the contribution of spices and animal fat to the formation of HCAs in barbecued sucuk and reported that the highest concentration of individual HCAs was 5.96 ng/g. They attributed the reason of low total HCA content in their samples to the presence of clove and cinnamon as spices. In a similar study, according to Murkovic et al. [33], spices can decrease the HCA amount in roasted meat.

There have been many studies about the determination of HCA content of meat products. To the best of our knowledge, there was no study about HCA formation in pan-fried sucuk samples, which were produced in Turkey. There is only one study on the formation of HCA in sucuk, which investigated the effects of different spices and fat types by Unal et al. [16], and in that study the presence or concentration of harman and norharman was not studied. However, in our study, we aimed to close the gap in literature by taking into account different cooking methods for sucuk preparation

and also the presence or formation of different HCAs in sucuk samples. Therefore, we aimed to make a survey about HCA content of sucuk samples produced and marketed in Turkey for the first time and also to investigate the effect of cooking temperature for pan frying on HCA content of sucuk samples.

Total HCA content of sucuk samples were lower compared to the most of the meat products. It could be related with chemical composition and raw materials (spices, food additives etc.) of sucuk. And also pan frying was used for cooking of sucuk samples. According to the results of different studies by using this cooking method, lower total HCA content is obtained compared to oven roasting and charcoal-barbecue.

According to Szterk [34], it was demonstrated that HCAs formation strongly correlates with the presence of various amino acids in raw beef as well as that of glucose and protein (correlation coefficient 0.84–0.93). Protein is precursor substance of HCA formation. Therefore, it is important to determine soluble protein content of sucuk samples. Soluble protein content of sucuk samples cooked at different temperatures were given in Table 4. Soluble protein content of samples were found between 19.00 mg/100 g and 57.60 mg/100 g; 23.90 mg/100 g and 41.80 mg/100 g; 24.40 mg/100 g and 53.20 mg/100 g for rare, medium and over-cooked samples, respectively. Statistically significant differences were found between soluble protein content of rare, medium and over cooked sucuk samples ($P<0.05$).

Table 4. Soluble protein content (mg/100 g) of cooked sucuk samples (Mean ± standard deviation)

Samples	Rare cooked	Medium cooked	Over cooked
S1	33.90^{d}±1.70	33.80^{cd} ±1.50	37.00^{c}±0.60
S2	53.61^{b} ±1.81	32.92^{d} ±1.33	40.31^{b}±0.60
S3	24.60^{f} ±0.80	41.00^{a} ±1.90	33.10^{d}±1.90
S4	31.63^{e} ±2.14	34.00^{cd} ±2.10	37.12^{c}±0.70
S5	53.10^{b} ±1.00	41.80^{a} ±1.50	26.60^{ef}±0.60
S6	37.40^{c}±0.60	24.50^{f} ±3.44	51.90^{a}±3.30
S7	57.60^{a} ±1.40	38.30^{b} ±0.40	29.50^{e}±0.80
S8	19.00^{g} ±1.30	23.90^{f} ±1.70	27.90^{e} ±0.90
S9	36.34^{c}± 3.92	29.00^{e} ±0.60	24.40^{f} ±2.50
S10	22.90^{f} ±1.50	36.40^{bc} ±1.74	53.20^{a}±1.13

[a] Different matching letters in the same column mean significant differences between the samples according to Duncan test ($P < 0.05$), All the analyses were performed triplicate

Canlı [35] determined total HCA content, moisture content and soluble protein content of 10 beef meatball samples. According to Pearson correlation test, positive correlation was obtained between moisture content and MeIQx content; norharman content; PhIP content of meatball samples ($P<0.01$). Positive correlation was obtained between soluble protein content and PhIP content of meatball samples ($P<0.01$). Correlations between chemical characteristics and heterocyclic amines were given in Table 5.

Choi et al. [36] determined soluble protein content of dried pork products. Total soluble protein content of low, medium, high protein containing samples were determined as 204.92 mg/kg, 184.43 mg/kg, and 142.33 mg/kg, respectively. Canlı [35] found soluble protein content of beef meatball between 1.28 mg/kg and 7.94 mg/kg; beef döner between 2.85 mg/kg and 8.33 mg/kg. Total soluble protein content of sucuk samples were higher than the literature values. Sucuk is a fermented product therefore; this could affect total soluble protein content of product.

Table 5. Correlations between chemical characteristics and individual and total heterocylic aromatic amine content of sucuk

	Moisture content	Crude fat content	pH	Crude protein content	Ash content	Total HCA 1	Total HCA 2	Total HCA 3
IQ (1)	▲	■	Δ	Δ	Δ	▲	Δ	Δ
4,8-DiMeIQx (1)	▲	■	Δ	Δ	Δ	▲	Δ	Δ
Norharman (1)	Δ	Δ	Δ	Δ	Δ	▲	Δ	Δ
Total HCA (1)	Δ	Δ	Δ	Δ	Δ		Δ	Δ
IQ (2)	▲	■	Δ	Δ	Δ	Δ	▲	Δ
4,8-DiMeIQx (2)	▲	■	Δ	Δ	Δ	Δ	▲	Δ
Norharman (2)	Δ	Δ	Δ	Δ	Δ	Δ	▲	Δ
Total HCA (2)	Δ	Δ	Δ	Δ	Δ	Δ		Δ
IQ (3)	▲	Δ	Δ	Δ	Δ	Δ	Δ	Δ
4,8-DiMeIQx (3)	▲	Δ	Δ	Δ	Δ	Δ	Δ	Δ
Norharman (3)	Δ	Δ	▲	▲	▲	Δ	Δ	▲
Total HCA (3)	Δ	Δ	▲	Δ	Δ	Δ	Δ	

▲ means significant positive correlation, ■ means significant negative correlation, Δ means no significant correlation. (1) means rare cooked, (2) means medium cooked (3)over cooked. Correlations were determined by Pearson correlation test at $P<0.05$ level

CONCLUSION

We have studied the formation and detection of HCAs in pan-fried sucuk. Norharman, which was the most abundant HCA in sucuk, was detected up to 10.54 ng/g in over-cooked sucuk samples, followed by PhIP with a concentration up to 9.75 ng/g, then 4,8-DiMeIQx up to 5.98 ng/g, and lastly IQ up to 3.84 ng/g. Total HCA

content of sucuk samples were found between 1.52 ng/g and 17.89 ng/g. HCA was quantified in most of the analyzed sucuk samples. Over-cooked sucuk samples had the maximum total HCA concentration, whereas rare-cooked ones had the lowest.

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