separations

MDPI

Article

Impact of Cumin (*Cuminum cyminum*) Incorporation on the Generation of Heterocyclic Aromatic Amines in Meatballs

Elif Ekiz [1], Adem Savaş [1], Eyad Aoudeh [1], Zeynep Elbir [1], Emel Oz [1], Charalampos Proestos [2], Naushad Ahmad [3] and Fatih Oz [1,*]

[1] Department of Food Engineering, Agriculture Faculty, Atatürk University, 25240 Erzurum, Türkiye
[2] Laboratory of Food Chemistry, Department of Chemistry, School of Sciences, National and Kapodistrian University of Athens, Zografou, 15784 Athens, Greece; harpro@chem.uoa.gr
[3] Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
* Correspondence: fatihoz@atauni.edu.tr

Abstract: In the current study, the impacts of using cumin (0.5% and 1%, *w*/*w*) in beef meatballs on heterocyclic aromatic amines (HAAs) formation and some quality characteristics when cooked at 150 and 250 °C were investigated. It was found that using of cumin at different ratios in beef meatballs showed a significant ($p < 0.01$) effect on pH, thiobarbituric acid reactive (TBARS) value, and total HAA. The cooking process significantly ($p < 0.01$) affected the meatballs' water content, pH, and TBARS values, while the cooking loss and total HAA content of meatballs were significantly ($p < 0.01$) affected by cooking temperature. It was found that the cumin usage rate in meatballs increased the pH value, while it was found to decrease the TBARS value. As expected, the cooking process was associated with a reduction in water, while it was found to result in an increase in the pH and TBARS values. On the other hand, both cooking temperature and cumin addition to meatballs led to an increase in their total HAA content. Diverse samples exhibited detectable levels of IQx, MeIQx, MeIQ, and PhIP compounds. The presence of MeIQx compound was found in all samples, except for the control group cooked at 150 °C. Additionally, the use of 0.5% and 1% cumin in meatballs cooked at temperature of 250 °C had an inhibitory effect on MeIQx compound. Our results revealed that the incorporation of cumin in the meatballs resulted in an increase in the total HAA content, likely due to its prooxidant effect. However, it was found that the use of cumin at certain rates could reduce the formation of MeIQx compound with an antioxidant effect. However, in this study found that even if 100 g of meatballs with the highest concentration of total HAA were consumed, the maximum HAA exposure limit value (0–15 μg/day) was not exceeded.

Keywords: meatball; cumin; cooking; heterocyclic aromatic amine; quality criteria; HPLC-DAD

Citation: Ekiz, E.; Savaş, A.; Aoudeh, E.; Elbir, Z.; Oz, E.; Proestos, C.; Ahmad, N.; Oz, F. Impact of Cumin (*Cuminum cyminum*) Incorporation on the Generation of Heterocyclic Aromatic Amines in Meatballs. *Separations* **2023**, *10*, 458. https://doi.org/10.3390/separations10080458

Academic Editors: Javier Saurina and Victoria Samanidou

Received: 26 June 2023
Revised: 31 July 2023
Accepted: 16 August 2023
Published: 21 August 2023

1. Introduction

Meat is considered an essential food in human nutrition because of its major components, such as protein and fat, and minor components, such as vitamins and minerals. Meat is usually cooked or processed into various products to be suitable for human consumption. The processes used for this purpose cause some changes in the meat, and the nutritional value, texture, and organoleptic properties of the meat are positively or negatively affected depending on the process used [1]. In fact, lipid oxidation, which occurs as a result of contact with air of unsaturated fatty acids during various pretreatments such as mincing, cooking, or transformation into products, has the property of being one of the reactions that have a very important impact on health and quality [2]. The use of antioxidant substances to protect foods from oxidative deterioration comes to the fore among the methods applied [3].

On the other hand, due to their carcinogenic and toxic properties, synthetic antioxidants have been replaced by the search for antioxidant substances found in the natural

structures of food or medicinal materials [4,5]. It is known that Maillard reaction products, phospholipids, sterols, and antioxidant compounds found in the structures of various vegetables, fruits, and seeds have an effect on reducing oxidative damage in many diseases such as cardiovascular disease, cataracts, arthritis, diabetes [5,6]. Food phenolics mainly have antioxidant activity as singlet oxygen quenchers, reducing agents and hydrogen donors. Some phenolics have chelating effects on metal ions that catalyze oxidation reactions. Through complex formation with catalytic metal ions, flavonoids exhibit the ability to scavenge and neutralize diverse free radicals (peroxide, hydroxyl, and superoxide radicals). In addition, flavonoids are also known to prevent oxidative rancidity in foods by inhibiting the enzymes lipoxygenase and cyclooxygenase. In this context, herbs and spices are known for their high antioxidant content and have been safely consumed for centuries [6].

Spices, which have an extensive history of utilization and have functions such as preserving foods, prolonging their preservation period, and adding color, taste, and aroma to food items, have been employed for therapeutic purposes in folk medicine for centuries [7–9]. Many spices are known to have significant anticarcinogenic, antioxidant, and antimutagenic effects [8,10]. In this direction, cumin (*Cuminum cyminum*), which ranks among the most extensively used spices, belongs to the Apiaceae family and contains 2.5–6% essential oil, 10–23% oil, 15–25% protein, in addition to varying proportions of tannin, flavonoids, resin, and gum [9–11]. It has been reported that antioxidant substances such as cuminal, pinocarveol, γ-terpinene, carotol and linalool 1-methyl-2- (1-methylethyl) benzene isolated from cumin have free radical scavenging and metal chelating effects [6]. In addition, studies have shown that the polyphenols found in cumin have antioxidant properties and it has been emphasized that they are good free radical scavengers [4,7]. By cooking of meat, some compounds that have been proven to be mutagens and/or carcinogens by epidemiological studies are generated. Heterocyclic aromatic amines (HAAs) occupy an important position among these compounds [12,13]. HAAs are chemical substances occurred throughout the cooking of protein-rich foods at temperatures higher than 150 °C [14,15]. HAAs, which were initially identified in meat and fish in 1977 [16], are divided into two main chemical classes according to their formation temperatures [13]. These are thermal (aminoimidazoazoarenes) and pyrolytic (aminocarbolines) HAAs. Aminoimidazoazoarenes are occurred during reactions in-volving creatine, creatinine, amino acids and reducing sugars (hexoses) at temperatures ranging from 150 °C to 300 °C, whereas aminocarbolines are typically generated through the pyrolysis of amino acids and proteins at temperatures above 300 °C [17–19]. In the light of epidemiological studies, it has been determined that the majority of HAAs exhibit mutagenic properties and nearly all of them are carcinogenic [20]. It is stated that there is a correlation between pancreatic, colorectal and breast cancers and high consumption of fish and meat, and a large part of this positive correlation is due to HAAs [15,21]. The cooking conditions (time, equipment, temperature, and method), meat type, pH, lipid oxidation, fats, heat transfer, mass transfer, carbohydrates, water activity, creatine, amino acids, and antioxidant substances are effective on the types and amounts of generation of HAAs [12,22].

Extensive research in the literature has focused on the formation of HAAs [23–26]. Recently, studies have progressed in the direction of the factors influencing the generation of HAAs and the reduction of their generation. In this context, the impacts of several spices on HAA generation have been investigated. The number of studies with various spice additions is quite high in the literature [27–30]. On the other hand, as far as we know, there is just a single study in the literature investigating the impact of cumin on the generation of HAAs [31]. In their study, Puangsombat et al. [31] examined the impact of both single concentration and single temperature on HAA formation, and fewer HAA compounds were investigated compared to the current research. Therefore, in the current study, the impact of using cumin in meatballs on the generation of HAA was investigated and it was aimed to consolidate the data obtained with the previous valuable study on this subject and to obtain more data. Meatballs with different amounts of cumin added (0.5% and 1%) were cooked at two temperatures (150 °C and 250 °C) and the amount and type of HAA

were investigated. In addition, various quality criteria of meatballs in both raw and cooked forms were determined.

2. Materials and Methods

2.1. Material

In this research, the beef round muscle (M. *Gluteus medius*) and intermuscular fat were acquired from the local butcher (Erzurum, Türkiye), while cumin was obtained from a local market (Erzurum, Türkiye). The muscle and the intramuscular fat were obtained from two different male cattle and used raw material. In the selection of these materials, care was taken to use animal meats of the same race, gender, and age. At 24 h after slaughter, these raw materials were delivered to the laboratory under controlled cold chain. After the connective tissues and visible fat of the supplied meat were removed, they were processed through grinding to produce minced meat, which was then employed in the preparation of meatball dough. Then, meatball dough was prepared at the rate of 15% fat from intermuscular fat obtained from the same carcass as the meat minced by the Babcock method. After dividing the meatball dough into 3 equivalent pieces, a group selected depending on chance was evaluated as a control group, while the remaining 2 groups were mixed by adding 0.5% and 1% cumin. In the current study, in the selection of M. *Gluteus medius* muscle in meatball production, it was taken into consideration that its chemical composition is suitable for meatballs and that this muscle is generally preferred in studies. In determining the concentrations of cumin, other studies examining the impact of spices and their extracts on HAA generation were taken into consideration [32–35].

The homogenized meatball doughs were kept at 4 °C for 6 h and then shaped in 7 × 1 cm dimensions by means of a steel mold. In the production of meatballs, no other additives and/or spices were used to prevent any interaction on the analyses.

2.2. Method

2.2.1. Cooking Conditions

An electric hot plate (Hot Plate, Test Laboratory Equipment, 460C, İstanbul, Türkiye), was utilized to cook the meatballs at two temperatures (150 °C and 250 °C) for a total of 8 (4 + 4) min. No fats/oils or water were used in the cooking process. Once cooled to room temperature, the meatballs were homogenized by a blender. For the HAA analysis, homogenized meatball samples were stored (−18 °C) [26].

2.2.2. Determination of the Water Content

The percentage of water in meatballs was founded by drying 10 g of sample weighed in the drying cups in an oven (Redline, RE53, Binder, Germany) at 100 °C (approximately 1 night) [36].

2.2.3. Determination of Cooking Loss

The meatballs, which had been weighed prior to cooking, were cooled to approximately 25 °C after cooking on the hot plate and weighed, and the cooking losses were determined by calculating the difference in % [37].

2.2.4. Determination of pH Value

The meatball sample (10 g) was homogenized along with 100 mL of distilled water in Ultra-Turrax (IKA T18, Staufen, Germany) for 1 min and then pH (Mettler Toledo 420, Greifensee, Switzerland) was measured [36].

2.2.5. Determination of TBARS

The TBARS analysis was conducted to measure the degree of lipid oxidation. For this purpose, of trichloroacetic acid solution (12 mL) was homogenized (Ultra-Turrax, IKA T18, Staufen, Germany) with of sample (2 g) and filtered through filter paper (Whatman No:1, Amersham, UK). After mixing the filtrate (3 mL) with thiobarbituric acid solution (0.02 M,

3 mL), they were kept in A water bath set at boiling temperature (40 min) and centrifuged (Hettich, EBA20, Tuttlingen, Germany). After centrifugation, absorbance measurement (532 nm) was performed in spectrophotometer (Shimadzu, UV 120-01, Kyoto, Japan). The results of the analysis are given in malondialdehyde (MDA)/kg [38].

2.2.6. Determination of HAAs

After combining the meatball sample (1 g) with 12 mL of NaOH (1 M) and stirring for 1 h, the Extrelut NT packaging material (10 g) was placed on it and mixed thoroughly and packaging was done with an Oasis MCX cartridge. Afterwards, the extraction was done out with ethyl acetate (75 mL), methanol (2 mL), and HCl (0.1 N, 2 mL), and in the last step, the extract was eluted with 2 mL of methanol:ammonia (NH_3, 32%) (19:1). The obtained extract was stored at −18 °C until High Performance Liquid Chromatography (HPLC) analysis. Prior to the analysis, the extract was placed in an oven at 45 °C overnight to eliminate methanol:NH_3, and subsequently, methanol (100 µL) containing the internal standard (4,7,8-TriMeIQx) was added for analysis using the HPLC-Diode Array Detector system [39]. The incorporation of an internal standard (4,7,8-TriMeIQx, 1 ng/mL) was intended to increase the precision and accuracy of the obtained results and to understand whether there was a shift in the other HAAs' peaks or not. In HPLC analysis, the injection volume was determined as 10 µL. In this study, the levels of HAAs present in the samples were measured by conducting standards at different concentrations. The identification of HAAs involved a comparison of their retention times in the samples and the retention times of HAAs whose UV spectra are known as standard. For the quantitative determination of HAAs, the external calibration curve method was employed. The basis for determining the recovery was the standard addition method. For this purpose, a mix stock solution at concentrations of 1, 2.5, 5 and 7.5 ng/g was added to the raw sample before extraction. The peak area against the amount of analyte added was drawn as curve. The recoveries were calculated based on the ratio between the slopes of the linear regression lines for the standard addition and the HAA standard solutions. HPLC conditions (Table 1) for the HAA analysis were referenced by Oz et al. [40].

Table 1. HPLC conditions for the HAA analysis.

HPLC	Thermo Ultimate 3000, Thermo Scientific, USA (Germering, Germany)
Diode Array Detector	DAD-3000
Autosampler	WPS-3000
Column Oven	TCC-3000
Pump	LPG-3400SD
Flow Rate	0.7 mL/min
Column	Acclaim™ 120 C18, 3 µm (4.6 × 150 mm)
Solvent A	Methanol/Acetonitrile/Water/Acetic acid (8/14/76/2, *v*/*v*/*v*/*v*) (pH 5.0)
Solvent B	Acetonitrile
Gradient Program	0% B, 0–10 min; 0–23% B, 11–20 min; 23% B, 21–30 min; 0% B, 31–45 min

2.2.7. Statistical Analysis

By adopting the Randomized Complete Block Design, the study was designed and implemented with two replications. Evaluation of the results was carried out via the SPSS package program (IBM SPSS version 25), and the differences between the outcomes were investigated through the application of the Duncan multiple comparison test.

3. Results

3.1. Water Content, pH, and TBARS Results of Meat Muscle, Intermuscular Fat, and Meatball Dough

The quality of meat and meat products relies on essential parameters such as water percentage, pH value, and lipid oxidation (TBARS value), all of which can be impacted by the cooking process. In addition, they can be related for the formation of HAAs [41,42]. The results of water percentage, pH and TBARS values of meat muscle, intermuscular fat,

and meatball dough, were presented in Table 2. As can be presented in Table 2, water, pH, and TBARS values were determined as 74.70%, 5.46, and 0.31 mg MDA/kg in beef round meat; 13.89%, 6.47, and 0.38 mg MDA/kg in (intermuscular) meat fat; and 65.62%, 5.63, and 0.34 mg MDA/kg in raw meatballs, respectively (Table 2). There are similar results in the literature [32,35].

Table 2. Water percentage, pH and TBARS values of beef, intermuscular fat and meatball dough with 15% fat.

	n	Water (%)	pH	TBARS (mg MDA/kg)
Meat	2	74.70 ± 0.18 a	5.46 ± 0.01 b	0.31 ± 0.04 a
Intermuscular fat	2	13.89 ± 0.16 c	6.47 ± 0.37 a	0.38 ± 0.13 a
Meatball dough	2	65.62 ± 0.8 b	5.63 ± 0.08 b	0.34 ± 0.07 a
Sign.		**	*	ns

a–c: Different letters indicate significant differences in column; ns: Not significant ($p > 0.05$); Sign.: Significant; * $p < 0.05$; ** $p < 0.01$.

3.2. Water Percentage, pH, TBARS, Cooking Loss, and HAA Results of All Meatball Samples

In the present study, the average water percentage, pH, TBARS, cooking loss, and total HAA results of all meatball samples based on the cumin usage rate (0, 0.5, and 1%), cooking process (raw and cooked) and cooking temperature (150 and 200 °C) were given in Table 3. The usage rate effect covers the cooking process (raw and cooked meatballs, n = 2), cooking temperatures (150 and 200 °C, n = 2) and replication (n = 2). Therefore, the number of samples was 8. The cooking process effect covers the cumin usage rate (0%, 0.5% and 1%, n = 3), cooking temperatures (150 and 200 °C, n = 2) and replication (n = 2). Therefore, the number of samples was 12. On a different note, the influence of cooking temperature influence covers the cumin usage rate (n = 3), cooking process (raw and cooked meatballs, n = 2) and replication (n = 2). Therefore, the number of samples was 12.

Table 3. The average water percentage, pH, TBARS, cooking loss values and total HAA contents of the meatball.

	Water (%)	pH	TBARS (mg MDA/kg)	Cooking Loss (%)	Total HAA (ng/g)
		Usage rate (UR, %)			
0	57.05 ± 6.44 a	5.73 ± 0.13 b	1.03 ± 0.27 a	43.81 ± 3.23 a	0.33 ± 0.43 b
0.5	58.45 ± 7.94 a	5.92 ± 0.06 a	0.63 ± 0.15 b	45.74 ± 0.79 a	1.20 ± 0.71 a
1	58.45 ± 7.67 a	5.95 ± 0.06 a	0.67 ± 0.19 b	44.29 ± 1.78 a	1.11 ± 1.00 a
Sign.	ns	**	**	ns	**
		Cooking Process (CP)			
Raw	64.51 ± 2.26 a	5.81 ± 0.14 b	0.60 ± 0.14 b	-	-
Cooked	51.45 ± 2.60 b	5.93 ± 0.08 a	0.96 ± 0.26 a	-	-
Sign.	**	**	**		
		Cooking Temperature (*CT*, °C)			
150	58.56 ± 6.49 a	5.86 ± 0.13 a	0.77 ± 0.29 a	43.05 ± 1.96 b	0.30 ± 0.29 b
250	57.40 ± 7.88 a	5.87 ± 0.13 a	0.78 ± 0.27 a	46.18 ± 0.66 a	1.46 ± 0.69 a
Sign.	ns	ns	ns	**	**

a, b: Different letters indicate significant differences in column; ns: Not significant ($p > 0.05$); Sign.: Significant; ** $p < 0.01$.

3.2.1. Water Percentage in Meatballs

The percentage of water in raw and cooked meatball samples are presented in Table 3. Our results revealed that the cooking process exhibited a statistically significant impact ($p < 0.01$) on the water percentage beef meatballs, whereas the incorporation of cumin at various ratios and the cooking level (temperature) did not show statistically significant effect ($p > 0.05$). It was determined that the water content of raw meatballs decreased as expected with the cooking process. It is known that the water content of the meat decreases

with the cooking process due to the evaporation of the free water content of the meat and the changes in the connective tissue and proteins [43]. Similar changes in water content due to the effect of cooking have been demonstrated by other researchers [28,44].

3.2.2. Cooking Loss Results of the Meatballs

Cooking loss in meat and meat products is a consequential alteration arising from the cooking process and holds significant economic importance. The color, flavor, juiciness, tenderness, vitamin and mineral levels of the meat are correlated with cooking time and cooking losses [45]. The cooking loss results of raw and cooked meatball samples are presented in Table 3. The cooking temperature had a statistically very significant impact ($p < 0.01$) on the cooking loss of meatballs, while the use of cumin at different ratios did not show a statistically significant impact ($p > 0.05$). It has been reported that most of the mass loss caused by cooking of meat is in the form of water loss as a result of denaturation and shrinkage, which take places in proteins as a consequence of temperature elevation. As a matter of fact, cooking conditions such as time and temperature along with the specific attributes of the meat such as meat component, size, and pH value, contribute to the variation in cooking loss [45,46]. Gerber et al. [47] reported that water removed from meat by the cooking process may also contain other water-soluble components such as proteins, collagen, lipids, polyphosphates, salt, and flavor compounds. While no significant difference was observed in meatball samples with cumin added at different rates compared to the control group, an increment in cooking losses was determined in meatball samples prepared at various temperatures. In this regard, the increase in cooking temperature has an increasing impact on the values of cooking loss. There are studies in the literature with similar results regarding cooking loss in various meat products cooked by different methods [23,46,48,49].

3.2.3. pH Values of the Meatballs

In the current study, the pH values of raw and cooked meatball samples are given in Table 3. While it was determined that the use of cumin in different rates and the cooking process had a very significant effect ($p < 0.01$) on the pH value of the meatballs, no statistically significant effect ($p > 0.05$) was observed in terms of the cooking temperature. A statistically significant increase in the pH values was detected of meatball samples to which different amounts of cumin were added compared to the control group. This increase is thought to be related to the pH value of cumin (mean 5.93 ± 0.03). The cooking process caused an increase in the pH value of the meatballs, as expected. Elbir et al. [26] explained the pH increase in meat after cooking with the release of bonds containing imidazole, hydroxyl, and sulfhydryl groups in meat with cooking. It was detected that the cooking temperature did not cause a significant change in the pH value. This result can be explained by the fact that the bonds containing sulfhydryl, imidazole, and hydroxyl groups in meat become sufficiently free even at 150 °C. Similar results on changes in pH have been put forward by other researchers [1,50].

3.2.4. TBARS Values of the Meatballs

TBARS analysis is the common a method used to measure secondary oxidation products that cause oxidative rancidity [2]. Oxidation reactions cause the formation of harmful compounds as well as affecting the quality criteria such as shelf life and nutritional value of the products [51]. For this reason, TBARS analysis is among the important quality parameters in meat and meat products. The TBARS values of raw and cooked meatball samples are given in Table 3. It was found that the use of cumin had a very significant effect ($p < 0.01$) on the TBARS value and there was a decrease in the TBARS values independent of the cumin ratio. It is thought that antioxidant substances such as γ-terpinene, linalool, pinocarveol, carotol, and 1-methyl-2-(1-methylethyl) benzene found in cumin are effective in reducing the TBARS value due to their free radical scavenging and metal chelating effects [6]. Heat treatment has been reported to disrupt heat-sensitive phenolic compounds

and changes their molecular structure in spice [52]. Gunasena and Rajapakse [53] reported that temperature and duration have positive effects on antioxidant activity in spices to some extent; however, there are losses in antioxidant activity as the duration is extended. It is considered that the changes in TBARS values depending on the cumin ratio are influenced by the temperature degradation of the antioxidant compounds contained in cumin. It has been reported that phenolic compounds prohibit the formation and the spread of free radical reactions in various ways, and the products formed in the first steps of the Maillard reaction, which occurs in meat at cooking temperatures above 90 °C, show antioxidant properties with free radical scavenging activity [54,55]. It was observed that the cooking process had a very significant effect ($p < 0.01$) on the TBARS value and the cooking process leaded to a significant increase in the TBARS value of the samples. It has been shown that oxidation reactions are affected by various factors (light, temperature, oxygen, metals, radiation, and the presence of antioxidant substances) [56]. However, it is also stated that heat treatment is more important than other factors in increasing the lipid oxidation of the samples. The increase in TBARS value seen with the cooking process is due to the increase in the level of malondialdehyde due to the effect of temperature, which is one of the important factors that accelerate oxidation [57]. In addition, the increase in TBARS value after cooking could be due to the release of polyunsaturated fatty acids and iron from myoglobin and hemoglobin compounds [35]. It was determined that cooking of the meatballs at different temperatures (150 °C and 250 °C) did not lead to significant change ($p > 0.05$) in the TBARS value. Lu et al. [28] reported that there may be an increase or decrease in TBARS values depending on the type of spice used in meatballs. Researchers attributed the decrease in TBARS value to the antioxidant substances found in the spices used and their activity. As a matter of fact, although there are studies showing that the TBARS value increased with the effect of cooking [49,50], there are also studies showing that it decreased [46]. On the other hand, it is stated that the acceptable TBARS value for meat products is a maximum of 3 mg MDA/kg, while values below this threshold do not pose a risk in terms of oxidative changes [57]. In this context, it was founded that the TBARS values detected in the control group and the meatball samples with cumin at different rates in the current study were below this limit.

3.2.5. Method Validation of HAAs

The standard addition method was preferred to calculate the recoveries of the HAAs examined in the study, and HAA mixture stock solutions at concentrations of 7.5, 5, 2.5 and 1 ng/g were added to the raw meatball samples before extraction. After the extraction and analysis performed for this purpose, the recoveries were determined. The LOD and LOQ values calculated according to the Signal/Noise (S/N) ratios from the HAA concentrations at certain concentrations are given in Table 4. The detected recoveries (81.52–95.64%) of the HAA compounds examined in this study are in agreement with the literature [39]. In addition, the chromatograms of the meatballs with the highest total HAA content and HAA mix stock solution are given in Figure 1.

Table 4. Recoveries, LOD, LOQ, linear equation and R^2 values (n = 4).

HAA	Recovery (%)	LOD (ng/g)	LOQ (ng/g)	Linear Equation	R^2
IQx (2-amino-3-methylimidazo[4,5-*f*]quinoxaline)	94.44	0.004	0.013	y = 2.3771x − 0.0111	0.9999
IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline)	82.17	0.009	0.029	y = 0.9659x − 0.0132	0.9999
MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline)	93.07	0.024	0.081	y = 1.5303x − 0.0103	0.9999
MeIQ (2-amino-3,4-dimethylimidazoimidazo[4,5-*f*]quinoline)	81.52	0.014	0.047	y = 0.8876x − 0.0195	0.9999
7,8-DiMeIQx (2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline)	92.96	0.005	0.018	y = 2.3739x − 0.0138	0.9999
4,8-DiMeIQx (2-amino-3,4, 8-trimethylimidazo[4,5-*f*]quinoxaline)	94.56	0.008	0.025	y = 1.841x − 0.0366	0.9999
PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine)	95.44	0.025	0.085	y = 0.168x + 0.0016	0.9999
AαC (2-amino-9H-pyrido [2,3-b]indole)	95.64	0.012	0.039	y = 0.4585x − 0.0036	0.9999
MeAαC (2-amino-9H-pyrido [2,3-b]indole)	90.53	0.010	0.035	y = 0.332x − 0.0167	0.9998

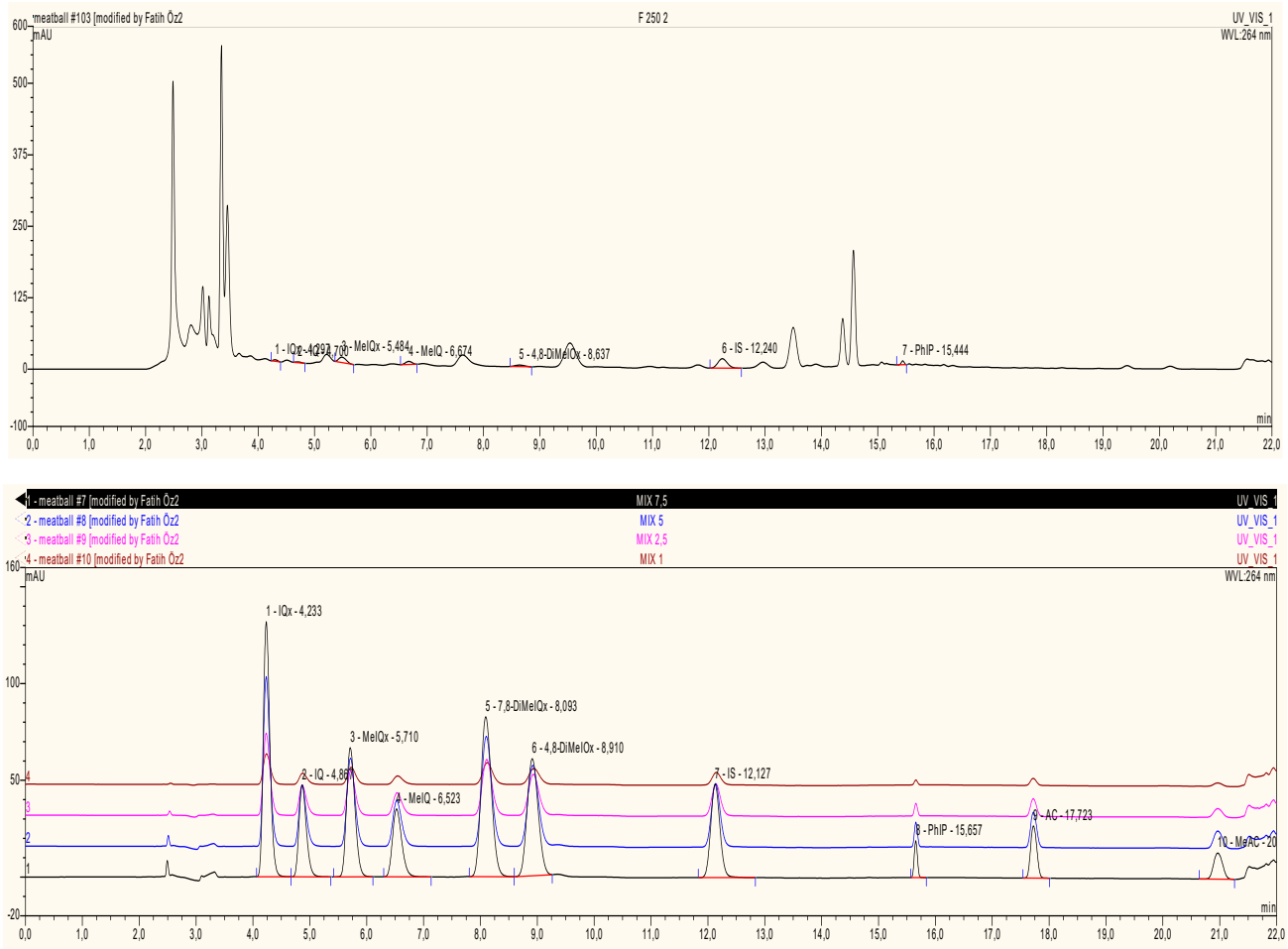

Figure 1. The chromatograms of the meatballs with the highest total HAA content and the HAA mix stock solution.

3.2.6. Total HAAs of the Meatballs

Total HAA content of cooked meatball samples are shown in Table 3. It was observed that cumin usage rate and cooking temperature had a statistically very significant effect ($p < 0.01$). While the total HAA content was detected as 0.33 ng/g in the control group, it was detected as 1.20 ng/g and 1.11 ng/g in the meatballs containing 0.5% and 1% cumin, respectively. The total HAA content was determined as 0.30 ng/g in the meatballs cooked at 150 °C and 1.46 ng/g in the meatballs cooked at 250 °C. It was determined that the total HAA content of the meatballs cooked at 150 °C and added cumin at the rate of 0.5% and 1% consisted of MeIQx and MeIQ compounds, while the total HAA content of the meatballs in the control group cooked at 250 °C consisted entirely of MeIQx compound. The total HAA content all of the meatballs increased in direct proportion to the temperature. It was observed that the use of cumin in meatballs increased the total HAA content compared to the control group, regardless of the rates added. It is thought that cumin, which was used in the meatball preparation in the present research, increased the content of many individual HAA and total HAA due to its pro-oxidant effect under the conditions in the research. Although it is known that the use of products with antioxidant effects has a reducing effect on HAA formation, there are also studies showing a prooxidant effect on the level of HAA formation. Johansson and Jägerstad [58] reported in their study that various antioxidants exhibited prooxidant effects on HAAs formation in model system. It was determined that HAA levels decreased in meats treated with various spice extracts (basil, coriander, thyme, marjoram, rosemary, savory, sweet herb, and thyme extracts) depending on cooking conditions and type of extract, but increased (PhIP) occurred in some samples depending on the

type of HAA. In addition, it was emphasized that heating conditions were more effective on HAAs than antioxidants in extracts [59]. Cumin contains antioxidant compounds such as γ-terpinene, linalool, quercetin, pinocarveol, carotol, 1-methyl-2-(1-methylethyl)benzene, ferulic acid, gallic acid, kaempferol, ellagic protocatechuic acid, caffeic acid and chlorogenic acid. Chlorogenic acid, one of the antioxidant compounds, can be converted into lactones of coumaroyl-caffeoyyl and feruloylquinic acids by heat treatment, as well as into various aromatic compounds consisting of proteins, carbohydrates, fats and aromatic acids [26,60]. Many factors are effective on types and amounts of HAA compounds, and compounds are formed by different mechanisms. While creatinine and alkylpyridine free radicals are effective in the formation of MeIQ and IQ compounds, dialkylprazine free radicals, as well as creatinine, are effective in the formation of 4,8-DiMeIQx and MeIQx compounds. It is also added that amino acids and Maillard and Strecker reactions are involved in MeIQx formation [56]. As a matter of fact, it has been reported that glycine, lysine, alanine, threonine, and serine play a role in the formation of MeIQx, and lysine, aspartic acid, isoleucine, and phenylalanine play a role in the formation of PhIP [61]. In this respect, the high carbohydrate (44.2%) and protein (17.8%) content of cumin provides the appropriate substrate conditions for the formation of the Maillard reaction during cooking [26,62].

Puangsombat et al. [31] cooked meatballs to which 0.2% cumin was added at 204 °C and examined the amounts of IQ, IQx, MeIQ, MeIQx and PhIP compounds from HAAs. The amount of MeIQx was determined as 6.94 ng/g, the amount of PhIP was determined as 6.14 ng/g, and the total HAA was determined as 13.08 ng/g. The researchers reported that inhibition of HAAs was dependent on the scavenging activity and total phenolic content of spices. As a matter of fact, there are studies examining the impacts of some spices, extracts, or fruits on the inhibition of HAAs [31,32,63]. Zhu et al. [64] emphasized that the inhibitory effect of antioxidant substances on HAAs is provided by the mechanism of clearance of intermediate products such as formaldehyde, such as Strecker aldehydes involved in the formation of MeIQx and IQ, and acetaldehyde involved in the formation of 4,8-DiMeIQx and MeIQ, rather than their free radical scavenging activities. Nuray and Oz [50] reported that antioxidant or prooxidant activities of antioxidant compounds can change depending on various parameters such as concentration, structure, substrate, and test method.

3.2.7. HAA Content of Meatballs

The HAA contents of cooked meatball samples are given in Table 5. In the present study, IQ, 7,8-DiMeIQx, 4,8-DiMeIQx, AαC and MeAαC could not be detected in any sample, while IQx, MeIQx, MeIQ, and PhIP were determined at varying levels in different samples.

Table 5. The individual HAA content of the meatball samples (ng/g).

Cooking Temperature (°C)	Usage Rate (%)	IQx	IQ	MeIQx	MeIQ	7,8-DiMeIQx	4,8-DiMeIQx	PhIP	AαC	MeAαC	Total HAA
150	0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	0.5	nd	nd	0.51	0.08	nd	nd	nd	nd	nd	0.59
	1	nd	nd	0.24	0.06	nd	nd	nd	nd	nd	0.30
250	0	nd	nd	0.91	nd	nd	nd	nd	nd	nd	0.91
	0.5	nd	nd	0.76	0.23	nd	nd	0.82	nd	nd	1.81
	1	0.08	nd	0.52	0.20	nd	nd	1.21	nd	nd	2.01

nd: not detected (<limit of detection).

In the meatball, IQx compound was detected only in the meatball samples with 1% cumin and cooked at 250 °C (0.08 ng/g). IQx compound, one of the less studied compounds in the literature, was detected up to 0.39 ng/g in pan-fried meat samples [65], up to 3.48 ng/g in a pan- and oven-cooked beef samples [32], and up to 3.06 ng/g in commercial beef samples [55]. It was stated that this compound could not be founded in meat samples [66], in meatball samples [67], and in burger samples [68]. Puangsombat et al. [31] stated that IQx compound was not detected in meatball samples they cooked for 10 min at 204 °C by adding cumin at a rate of 0.2%.

In this study, the amount of IQ compound in the meatballs was determined below the LOD value. It is stated that IQ compound was not founded in pan-fried beef and lamb chops [69]; in pan-fried beef meatballs [34]; and in meatball samples cooked on a hot plate [35]. IQ compound was not defined in meatball samples cooked (204 °C/10 min) by adding cumin at a rate of 0.2% [31].

MeIQx was founded in all meatball samples except the control group, which was cooked at 150 °C. There are studies in which the MeIQx compound has been detected [34,56] in various meat products and has not been detected [50]. It was determined that the addition of cumin increased in the MeIQx compound in meatballs cooked at 150 °C compared to the control group. However, increasing the cumin content from 0.5% to 1% in meatballs showed a reducing effect on the formation of MeIQx. It was determined that the increase in the cumin ratio in the meatball samples cooked at 250 °C compared to the control groups exhibited an inhibitory effect on MeIQx. In this context, it was determined that 0.5% cumin addition provided 16.48% inhibition, while 1% cumin addition provided 42.86% inhibition in the meatballs cooked at 250 °C. It has been stated that the addition of garlic to meatballs by Shin et al. [70], the addition of hibiscus extract to meatballs by Gibis and Weiss [71], the addition of red pepper to beef by Oz and Kaya [72], the addition of dried apple peel powder to meatballs by Sabally et al. [63], and the addition of black cumin to meatballs by Oz [49] exhibited inhibitory effects on MeIQx. Puangsombat et al. [31] stated that MeIQx compound was detected as 6.94 ng/g in meatball samples cooked at 204 °C by adding cumin at a rate of 0.2%, and a decrease in the amount of MeIQx compound was observed compared to the control group.

It was observed that the use of cumin increased MeIQx formation in the meatballs cooked at 150 °C compared to control group meatballs, and decreased MeIQx formation in the meatballs cooked at 250 °C. It is thought that this situation may be due to the fact that cumin shows both an antioxidant and a pro-oxidant effect depending on the cooking temperatures applied. Because, it is stated in the literature that antioxidant substances can have antioxidant and pro-oxidant effects depending on the ambient temperature [59,60].

MeIQ compound was below the LOD value in the control group meatballs cooked at 150 °C and 250 °C, while it was found between 0.06–0.23 ng/g in the meatballs with 0.5% and 1% cumin. In the meatballs cooked at 150 °C and 250 °C, the addition of cumin at a rate of 1% had an inhibitory effect on MeIQ compound compared to the addition of cumin at a rate of 0.5%. It has been reported that MeIQ compound was detected 0.1–3.5 ng/g in beef patties fried (175–225 °C and 12–20 min) by Balogh et al. [73]; 2.02–6.35 ng/g in beef cooked (150–250 °C and 10 min) by Tengilimoğlu-Metin and Kızıl [32]; 3.03–18.09 ng/g in fried meatball samples to which different spices were added by Lu et al. [28]. There are also studies in the literature in which MeIQ compound could not be founded [34,74]. Puangsombat et al. [31] stated that MeIQ compound was not detected in meatball samples cooked for 10 min at 204 °C by adding cumin at a rate of 0.2%.

7,8-DiMeIQx and 4,8-DiMeIQx compounds could not be defined in the meatballs. 7,8-DiMeIQx compound was detected up to 0.04 ng/g in meat samples with artichoke extract [32], and up to 1.36 ng/g in commercial meat samples [55]. 4,8-DiMeIQx compound was determined up to 1.19 ng/g in meat samples with artichoke extract [32], up to 11.31 ng/g in goat meatballs treated with Chrysanthemum morifolium extract [74], and up to 1.1 ng/g in meatball samples treated with various antioxidants [58]. There are also studies in which 7,8-DiMeIQx and 4,8-DiMeIQx compounds have not been detected [1,23,68].

PhIP compound could not be founded in the meatballs cooked at 150 °C and the control group meatballs cooked at 250 °C, while it was detected as 0.82 ng/g and 1.21 ng/g in the meatballs with cumin added at a rate of 0.5% and 1% and cooked at 250 °C, respectively. While PhIP compound was detected in meat samples up to 15.2 ng/g [65], the compound was determined in meatball samples up to 0.06 ng/g [71], up to 13.85 ng/g [28], up to 2.08 ng/g [74], and up to 3.1 ng/g [34]. Korkmaz and Oz [44] and Uzun and Oz [35] could not detect the PhIP compound in the meatball samples. PhIP compound was observed as 6.914 ng/g in meatball samples cooked at 204 °C for 10 min by adding cumin at a rate of

0.2%, and there was a decrease in the PhIP compound compared to the control group [31]. It has been revealed that PhIP compound is occurred as a result of the Maillard reaction between glucose, phenylalanine, and creatine. In the current study, increasing the cumin ratio in the meatballs cooked at 250 °C increased the amount of PhIP compound. It is thought that the fact that cumin contains 44.2% carbohydrates and 4.23% phenylalanine contributes to the formation of PhIP [60,62].

MeAαC and AαC compounds could not be founded in any of the meatball samples. Tengilimoglu-Metin and Kizil [32] stated that AαC compound ranged between nd—8.48 ng/g in the control group and meat samples to which artichoke extract was added; Khan et al. [74] reported that it ranged between 0.31–0.65 ng/g in control group goat meatballs and goat meatballs to which Chrysanthemum morifolium extract was added. Tengilimoglu-Metin et al. [23] stated that MeAαC compound changed between nd—0.48 ng/g in meat samples to which they added hawthorn extract and cooked at different temperatures. There are studies in the literature in which AαC and MeAαC compounds were not detected. It was stated that AαC and MeAαC compounds could not be determined in beef burgers [68], in meatball samples added with different onion extracts [50], in meatball samples in which black cumin was added at different ratios [49], and in commercially beef samples [55].

3.2.8. Correlation Analysis

The correlation analysis of all parameters examined in meatball samples is given in Figure 2. A strong and very significant positive correlation (r = 0.708; $p < 0.01$) was determined between total HAA content and cooking loss, while a strong and very significant negative correlation (r = −0.857; $p < 0.01$) was found between pH and TBARS values. The water percentage of the meat is important in transporting the precursors of the HAAs to the meat surface. During cooking, the substances that are removed with the water come to the outer surface of the meat and contribute to the formation of HAA [24]. Indeed, the negative correlation between water content and cooking loss confirms this situation. Therefore, increased cooking loss results in higher HAA content. In this study, the use of cumin in meatball samples increased the pH value. On the other hand, the use of cumin in meatballs decreased the TBARS value with its antioxidant effect. In this context, the negative correlation between TBARS and pH value is explained by the use of cumin.

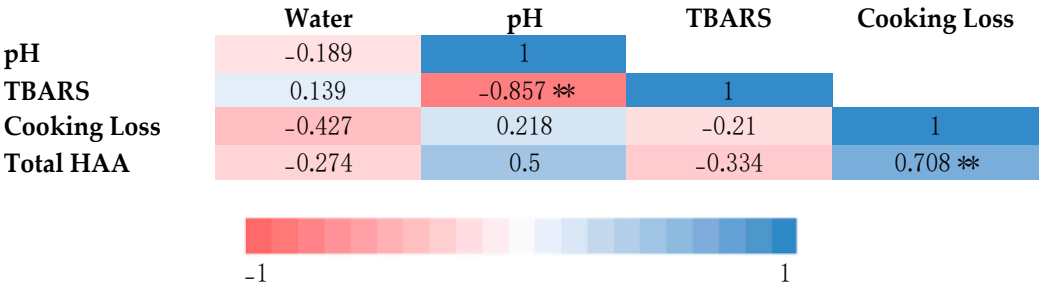

Figure 2. Correlation analysis of the meatballs. ** $p < 0.01$.

4. Conclusions

In the present study, the effect of cumin addition to meatballs cooked at two different temperatures (150 and 250 °C) on HAA formation was investigated. The results showed that the addition of cumin increased the formation of total HAA content in the meatballs compared to the control group meatballs. On the other hand, it has been reported that the maximum acceptable intake of heterocyclic aromatic amines (HAAs) is 0–15 µg/day [75], and even if 100 g of the meatballs with the highest total HAA content (samples with 1% cumin and cooked at 250 °C) were consumed in the current study, 0.201 µg of HAAs would be exposed. The inhibitory effect of cumin use in meatball production on HAAs was determined in MeIQx compound, and the highest inhibition (42.86%) belongs to meatballs cooked at 250 °C and added cumin at a rate of 1%. The phenolic compounds in

the structure of cumin are thought to inhibit HAA formation due to their antioxidant and radical scavenging effects. On the other hand, model system studies are needed to explain the clear mechanisms in this regard.

Author Contributions: Conceptualization, E.E. and F.O.; methodology, E.E. and F.O.; validation, E.E. and F.O.; formal analysis, E.E., A.S., Z.E., E.O., C.P., N.A. and F.O.; investigation, E.E. and F.O.; data curation, A.S., Z.E., E.O., C.P. and N.A.; writing—original draft preparation, E.E., A.S. and Z.E.; writing—review and editing, E.E., E.A., E.O., C.P., N.A. and F.O.; visualization, F.O.; supervision, F.O.; project administration, F.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available on reasonable request from the corresponding author.

Acknowledgments: The authors would like to thank National and Kapodistrian University of Athens for funding (grant no. 14053 and 15363). The authors would also like to thank the support provided by Ataturk University (Türkiye), National and Kapodistrian University of Athens (Greece), the Researchers Supporting Project number (RSD-2023R668), King Saud University (Riyadh, Saudi Arabia), for the preparation of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Oz, E. The presence of polycyclic aromatic hydrocarbons and heterocyclic aromatic amines in barbecued meatballs formulated with different animal fats. *Food Chem.* **2021**, *352*, 129378. [CrossRef] [PubMed]
2. Rahman, M.H.; Alam, M.S.; Monir, M.M.; Ahmed, K. Comprehensive effects of black cumin (*Nigella sativa*) and synthetic antioxidant on sensory and physicochemical quality of beef patties during refrigerant storage. *J. Agric. Food Res.* **2021**, *4*, 100145. [CrossRef]
3. Choe, E.; Min, D.B. Mechanisms of antioxidants in the oxidation of foods. *Compr Rev. Food Sci. Food Saf.* **2009**, *8*, 345–358. [CrossRef]
4. Sultana, S.; Ripa, F.A.; Hamid, K. Comparative antioxidant activity study of some commonly used spices in Bangladesh. *Pak. J. Biol. Sci.* **2010**, *13*, 340. [CrossRef] [PubMed]
5. Dua, A.; Gupta, S.K.; Mittal, A.; Mahajan, R. A study of antioxidant properties and antioxidant compounds of cumin (*Cuminum cyminum*). *Int. J. Pharm. Biol. Arch.* **2012**, *3*, 1110–1116.
6. Embuscado, M.E. Spices and herbs: Natural sources of antioxidants–A mini review. *J. Funct. Foods* **2015**, *18*, 811–819. [CrossRef]
7. Ani, V.; Varadaraj, M.C.; Naidu, K.A. Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (*Cuminum nigrum* L.). *Eur. Food Res. Technol.* **2006**, *224*, 109–115. [CrossRef]
8. Allahghadri, T.; Rasooli, I.; Owlia, P.; Nadooshan, M.J.; Ghazanfari, T.; Taghizadeh, M.; Astaneh, S.D.A. Antimicrobial property, antioxidant capacity, and cytotoxicity of essential oil from cumin produced in Iran. *J. Food Sci.* **2010**, *75*, 54–61. [CrossRef]
9. Devranbay, Ş. Kekikli Kimyonlu Beyaz Peynir Üretimi. Master's Thesis, Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Türkiye, 2016.
10. Berktaş, Ö.A. Kimyon, Tarçın ve Sumak Gibi Baharatların Ratlarda Antioksidan ve Antiülserojenik Özelliklerinin Belirlenmesi ve Biyokimyasal Olarak Incelenmesi. Ph.D. Thesis, Atatürk University, Erzurum, Turkey, 2017.
11. Başer, K.H.C. Kimyon (*Cuminum cyminum* L.). *Bağbahçe Derg.* **2014**, *55*, 26–27.
12. Oz, F.; Kızıl, M.; Zaman, A.; Turhan, S. The effects of direct addition of low and medium molecular weight chitosan on the formation of heterocyclic aromatic amines in beef chop. *LWT-Food Sci. Technol.* **2016**, *65*, 861–867. [CrossRef]
13. Zamora, R.; Hidalgo, F.J. Formation of heterocyclic aromatic amines with the structure of aminoimidazoazarenes in food products. *Food Chem.* **2020**, *313*, 126128. [CrossRef] [PubMed]
14. Chevolleau, S.; Bouville, A.; Debrauwer, L. Development and validation of a modified QuEChERS protocol coupled to UHPLC-APCI-MS/MS for the simple and rapid quantification of 16 heterocyclic aromatic amines in cooked beef. *Food Chem.* **2020**, *316*, 126327. [CrossRef] [PubMed]
15. Lee, S.Y.; Yim, D.G.; Kim, O.Y.; Kang, H.J.; Kim, H.S.; Jang, A.; Park, T.S.; Jin, S.K.; Hur, S.J. Overview of the effect of natural products on reduction of potential carcinogenic substances in meat products. *Trends Food Sci. Technol.* **2020**, *99*, 568–579. [CrossRef]
16. Oz, E.; Oz, F. Mutagenic and/or carcinogenic compounds in meat and meat products: Heterocyclic aromatic amines perspective. *Theory Pract. Meat Process.* **2022**, *7*, 112–117. [CrossRef]
17. Sugimura, T.; Adamson, R.H. Introduction. In *Food Borne Carcinogens*; Nagao, M., Sugimura, T., Eds.; JohnWiley & Sons Ltd.: West Sussex, UK, 2000; pp. 1–5.

18. Zamora, R.; Hidalgo, F.J. 2-Amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (PhIP) formation and fate: An example of the coordinate contribution of lipid oxidation and Maillard reaction to the production and elimination of processing-related food toxicants. *R. Soc. Chem.* **2015**, *5*, 9709–9721. [CrossRef]
19. Kang, H.J.; Lee, S.Y.; Kang, J.H.; Kim, J.H.; Kim, H.W.; Oh, D.H.; Jeong, J.W.; Hur, S.J. Main mechanisms for carcinogenic heterocyclic amine reduction in cooked meat by natural materials. *Meat Sci.* **2022**, *183*, 108663. [CrossRef] [PubMed]
20. Skog, K.I.; Johansson, M.A.E.; Jägerstad, M.I. Carcinogenic heterocyclic amines in model systems and cooked foods: A Review on formation, occurrence and intake. *Food Chem. Toxicol.* **1998**, *36*, 879–896. [CrossRef]
21. Khan, I.A.; Khan, A.; Zou, Y.; Zongshuai, Z.; Xu, W.; Wang, D.; Huang, M. Heterocyclic amines in cooked meat products, shortcomings during evaluation, factors influencing formation, risk assessment and mitigation strategies. *Meat Sci.* **2022**, *184*, 108693. [CrossRef]
22. Barzegar, F.; Kamankesh, M.; Mohammadi, A. Heterocyclic aromatic amines in cooked food: A review on formation, health risk-toxicology and their analytical techniques. *Food Chem.* **2019**, *280*, 240–254. [CrossRef]
23. Tengilimoglu-Metin, M.M.; Hamzalioglu, A.; Gokmen, V.; Kizil, M. Inhibitory effect of hawthorn extract on heterocyclic aromatic amine formation in beef and chicken breast meat. *Food Res. Int.* **2017**, *99*, 586–595. [CrossRef]
24. Savaş, A.; Oz, E.; Oz, F. Is oven bag really advantageous in terms of heterocyclic aromatic amines and bisphenol-A? Chicken meat perspective. *Food Chem.* **2021**, *355*, 129646. [CrossRef] [PubMed]
25. Zhang, L.; Wang, H.; Xia, X.; Xu, M.; Kong, B.; Liu, Q. Comparative study on the formation of heterocyclic aromatic amines in different sugar smoking time. *Food Control* **2021**, *124*, 107905. [CrossRef]
26. Elbir, Z.; Ekiz, E.; Aoudeh, E.; Oz, E.; Savaş, A.; Brennan, C.; Proestos, C.; Khan, M.R.; Elobeid, T.; Brennan, M.; et al. Enhancing effect of chia seeds on heterocyclic amine generation in meatball. *Int. J. Food Sci. Technol.* **2023**, *58*, 2560–2572. [CrossRef]
27. Jinap, S.; Iqbal, S.Z.; Selvam, R.M. Effect of selected local spices marinades on the reduction of heterocyclic amines in grilled beef (satay). *LWT-Food Sci. Technol.* **2015**, *63*, 919–926. [CrossRef]
28. Lu, F.; Kuhnle, G.K.; Cheng, Q. The effect of common spices and meat type on the formation of heterocyclic amines and polycyclic aromatic hydrocarbons in deep-fried meatballs. *Food Control* **2018**, *92*, 399–411. [CrossRef]
29. Kilic, S.; Oz, E.; Oz, F. Effect of turmeric on the reduction of heterocyclic aromatic amines and quality of chicken meatballs. *Food Control* **2021**, *128*, 108189. [CrossRef]
30. Savaş, A.; Ekiz, E.; Elbir, Z.; Savaş, B.D.; Proestos, C.; Elobeid, T.; Khan, M.R.; Oz, F. Advantageous Effects of Sumac Usage in Meatball Preparation on Various Quality Criteria and Formation of Heterocyclic Aromatic Amines. *Separations* **2023**, *10*, 29. [CrossRef]
31. Puangsombat, K.; Jirapakkul, W.; Smith, J.S. Inhibitory activity of Asian spices on heterocyclic amines formation in cooked beef patties. *J. Food Sci.* **2011**, *76*, T174–T180. [CrossRef]
32. Tengilimoglu-Metin, M.M.; Kizil, M. Reducing effect of artichoke extract on heterocyclic aromatic amine formation in beef and chicken breast meat. *Meat Sci.* **2017**, *134*, 68–75. [CrossRef]
33. Zeng, M.; Zhang, M.; He, Z.; Qin, F.; Tao, G.; Zhang, S.; Gao, Y.; Chen, J. Inhibitory profiles of chilli pepper and capsaicin on heterocyclic amine formation in roast beef patties. *Food Chem.* **2017**, *221*, 404–411. [CrossRef]
34. Meurillon, M.; Angenieux, M.; Mercier, F.; Blinet, P.; Chaloin, L.; Chevolleau, S.; Debrauwer, L.; Engel, E. Mitigation of heterocyclic aromatic amines in cooked meat. Part I: Informed selection of antioxidants based on molecular modeling. *Food Chem.* **2020**, *331*, 127264. [CrossRef] [PubMed]
35. Uzun, I.; Oz, F. Effect of basil use in meatball production on heterocyclic aromatic amine formation. *J. Food Sci. Technol.* **2021**, *58*, 3001–3009. [CrossRef] [PubMed]
36. Gökalp, H.Y.; Kaya, M.; Tülek, Y.; Zorba, Ö. *Et ve Ürünlerinde Kalite Kontrolü ve Laboratuvar Uygulama Klavuzu. (V. Baskı)*; Atatürk Üniv. Yayınları, Yayın No: 751, Ziraat Fak. Yayın No: 318; Ders kitapları seri no:69; Atatürk Üniversity Ofset Tesisi: Erzurum, Turkey, 2010.
37. Oz, F.; Kızıl, M. Determination of heterocyclic aromatic amines in cooked commercial frozen meat products by ultrafast liquid chromatography. *Food Anal. Methods* **2013**, *6*, 1370–1378. [CrossRef]
38. Kilic, B.; Richards, M.P. Lipid oxidation in poultry döner kebab: Pro-oxidative and anti-oxidative factors. *J. Food Sci.* **2003**, *68*, 686–689. [CrossRef]
39. Messner, C.; Murkovic, M. Evaluation of a new model system for studying the formation of heterocyclic amines. *J. Chromatogr. B* **2004**, *802*, 19–26. [CrossRef]
40. Oz, F.; Kizil, M.; Cakmak, I.H.; Aksu, M.I. The effect of direct addition of conjugated linoleic acid on the formation of heterocyclic aromatic amines in beef chops. *J. Food Process. Preserv.* **2015**, *39*, 2820–2833. [CrossRef]
41. Aksoy, A. Gıdalarda pH ölçümünün önemi. *Haliç Üniv. Fen Bil. Derg.* **2021**, *4*, 193–216. [CrossRef]
42. Sabuncular, G.; Akbulut, G.; Yaman, M. Ette lipit oksidasyonu ve etkileyen faktörler. *EJOSAT* **2021**, *27*, 362–369. [CrossRef]
43. Sánchez del Pulgar, J.; Gázquez, A.; Ruiz-Carrascal, J. Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Sci.* **2012**, *90*, 828–835. [CrossRef]
44. Korkmaz, A.; Oz, F. Effect of the use of dry breadcrumb in meatball production on the formation of heterocyclic aromatic amines. *Br. Food J.* **2020**, *122*, 2105–2119. [CrossRef]
45. Kondjoyan, A.; Oillic, S.; Portanguen, S.; Gros, J.B. Combined heat transfer and kinetic models to predict cooking loss during heat treatment of beef meat. *Meat Sci.* **2013**, *95*, 336–344. [CrossRef]

46. Serrano, A.; Librelotto, J.; Cofrades, S.; Sánchez-Muniz, F.J.; Jiménez-Colmenero, F. Composition and physicochemical characteristics of restructured beef steaks containing walnuts as affected by cooking method. *Meat Sci.* **2007**, *77*, 304–313. [CrossRef] [PubMed]
47. Gerber, N.; Scheeder, M.R.L.; Wenk, C. The influence of cooking and fat trimming on the actual nutrient intake from meat. *Meat Sci.* **2009**, *81*, 148–154. [CrossRef] [PubMed]
48. Domínguez, R.; Gómez, M.; Fonseca, S.; Lorenzo, J.M. Influence of thermal treatment on formation of volatile compounds, cooking loss and lipid oxidation in foal meat. *LWT-Food Sci. Technol.* **2014**, *58*, 439–445. [CrossRef]
49. Oz, E. Inhibitory effects of black cumin on the formation of heterocyclic aromatic amines in meatball. *PLoS ONE* **2019**, *14*, e0221680. [CrossRef] [PubMed]
50. Nuray, M.; Oz, F. The effect of using different types and rates of onion-water extract in meatball production on the formation of heterocyclic aromatic amines. *J. Sci. Food Agric.* **2019**, *99*, 3538–3547. [CrossRef]
51. Pereira, A.L.F.; Abreu, V.K.G. Lipid Peroxidation in Meat and Meat Products. In *Lipid Peroxidation Research*; Ahmed Mansour, M., Ed.; IntechOpen: London, UK, 2018; p. 29. [CrossRef]
52. Kumar, S.; Jain, I.; Khare, A.; Kumar, V.; Singh, S.; Gautam, P.; Upadhyay, S.; Anand, J.; Singh, A.; Goswami, U. Numerical optimization of microwave treatment and impact of storage period on bioactive components of cumin, black pepper and mustard oil incorporated coriander leave paste. *J. Food Meas. Charact.* **2022**, *16*, 2071–2085. [CrossRef]
53. Gunasena, B.V.A.P.; Rajapakse, R.P.N.P. Effect of Cooking Time and Cooking Temperature on Antioxidant Activity and Antimicrobial Activity of Cinnamon, Garlic, Ginger and Turmeric. In Proceedings of the Extended Abstracts of the 1st IFSTSL Research Session, Colombo, Sri Lanka, 15 December 2015; pp. 26–30.
54. Lepper-Blilie, A.N.; Berg, E.P.; Buchanan, D.S.; Keller, W.L.; Maddock-Carlin, K.R.; Berg, P.T. Effectiveness of oxygen barrier oven bags in low temperature cooking on reduction of warmed-over flavor in beef roasts. *Meat Sci.* **2014**, *96*, 1361–1364. [CrossRef]
55. Shan, S.; Ma, Y.; Sun, C.; Guo, X.; Zheng, H.; Xu, X.; Qin, L.; Hu, J. A novel magnetic solid-phase extraction method for detection of 14 heterocyclic aromatic amines by UPLC-MS/MS in meat products. *Food Chem.* **2021**, *337*, 127630. [CrossRef]
56. Ekiz, E.; Oz, F. The effects of different frying oils on the formation of heterocyclic aromatic amines in meatballs and the changes in fatty acid compositions of meatballs and frying oils. *J. Sci. Food Agric.* **2019**, *99*, 1509–1518. [CrossRef]
57. Weber, J.; Bochi, V.C.; Ribeiro, C.P.; Victório, A.D.M.; Emanuelli, T. Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. *Food Chem.* **2008**, *106*, 140–146. [CrossRef]
58. Johansson, M.A.E.; Jägerstad, M. Influence of pro- and antioxidants on the formation of mutagenic-carcinogenic heterocyclic amines in a model system. *Food Chem.* **1996**, *56*, 69–75. [CrossRef]
59. Damašius, J.; Venskutonis, P.R.; Ferracane, R.; Fogliano, V. Assessment of the influence of some spice extracts on the formation of heterocyclic amines in meat. *Food Chem.* **2011**, *126*, 149–156. [CrossRef]
60. Bouhenni, H.; Doukani, K.; Şekeroğlu, N.; Gezici, S.; Tabak, S. Comparative study on chemical composition and antibacterial activity of fenugreek (*Trigonella foenum graecum* L.) and cumin (*Cuminum cyminum* L.) seeds. *Ukr. Food J.* **2019**, *8*, 755–767. [CrossRef]
61. Du, H.; Wang, Z.; Li, Y.; Liu, Q.; Chen, Q.; Kong, B. Understanding the Development of heterocyclic aromatic amines in fried bacon and in the remaining oil after pan-frying in five different vegetable oils. *Foods* **2022**, *11*, 3491. [CrossRef] [PubMed]
62. Ceylan, E.; Özbek, H.; Ağaoğlu, Z. *Cuminum cyminum* L. (kimyon) meyvesi uçucu yağının median lethal doz (LD50) düzeyi ve sağlıklı ve diyabetli farelerde hipoglisemik etkisinin araştırılması. *Van Tıp Derg.* **2003**, *10*, 29–35.
63. Sabally, K.; Sleno, L.; Jauffrit, J.A.; Iskandar, M.M.; Kubow, S. Inhibitory effects of apple peel polyphenol extract on the formation of heterocyclic amines in pan fried beef patties. *Meat Sci.* **2016**, *117*, 57–62. [CrossRef]
64. Zhu, Q.; Zhang, S.; Wang, M.F.; Chen, J.; Zheng, Z.P. Inhibitory effects of selected dietary flavonoids on the formation of total heterocyclic amines and 2-amino-1-methyl-6-phenylimidazo 4,5-b pyridine (PhIP) in roast beef patties and in chemical models. *Food Funct.* **2016**, *7*, 1057–1066. [CrossRef]
65. Turesky, R.J.; Taylor, J.; Schnackenberg, L.; Freeman, J.P.; Holland, R.D. Quantitation of carcinogenic heterocyclic aromatic amines and detection of novel heterocyclic aromatic amines in cooked meats and grill scrapings by HPLC/ESI-MS. *J. Agric. Food Chem.* **2005**, *53*, 3248–3258. [CrossRef]
66. Pais, P.; Salmon, C.P.; Knize, M.G.; Felton, J.S. Formation of Mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats, and meat drippings. *J. Agric. Food Chem.* **1999**, *47*, 1098–1108. [CrossRef]
67. Oz, F.; Zaman, A.; Kaya, M. Effect of chitosan on the formation of heterocyclic aromatic amines and some quality properties of meatball. *J. Food Process. Preserv.* **2017**, *41*, e13065. [CrossRef]
68. Yan, Y.; Zhang, S.; Tao, G.J.; You, F.H.; Chen, J.; Zeng, M.M. Acetonitrile extraction coupled with UHPLC–MS/MS for the accurate quantification of 17 heterocyclic aromatic amines in meat products. *J. Chromatogr. B* **2017**, *1068*, 173–179. [CrossRef] [PubMed]
69. Öz, F.; Kaban, G.; Kaya, M. Heterocyclic aromatic amine contents of beef and lamb chops cooked by different methods to varying levels. *J. Anim. Vet. Adv.* **2010**, *9*, 1436–1440. [CrossRef]
70. Shin, H.S.; Rodgers, W.J.; Gomaa, E.A.; Strasburg, G.M.; Gray, J.I. Inhibition of heterocyclic aromatic amine formation in fried ground beef patties by garlic and selected garlic-related sulfur compounds. *J. Food Prot.* **2002**, *65*, 1766–1770. [CrossRef] [PubMed]
71. Gibis, M.; Weiss, J. Inhibitory effect of marinades with hibiscus extract on formation of heterocyclic aromatic amines and sensory quality of fried beef patties. *Meat Sci.* **2010**, *85*, 735–742. [CrossRef] [PubMed]

72. Oz, F.; Kaya, M. The inhibitory effect of red pepper on heterocyclic aromatic amines in fried beef Longissimus dorsi muscle. *J. Food Process. Preserv.* **2011**, *35*, 806–812. [CrossRef]
73. Balogh, Z.; Gray, J.I.; Gomaa, E.A.; Booren, A.M. Formation and inhibition of heterocyclic aromatic amines in fried ground beef patties. *Food Chem. Toxicol.* **2000**, *38*, 395–401. [CrossRef]
74. Khan, I.A.; Liu, D.; Yao, M.; Memon, A.; Huang, J.; Huang, M. Inhibitory effect of *Chrysanthemum morifolium* flower extract on the formation of heterocyclic amines in goat meat patties cooked by various cooking methods and temperatures. *Meat Sci.* **2019**, *147*, 70–81. [CrossRef]
75. Skog, K. Problems associated with the determination of heterocyclic amines in cooked foods and human exposure. *Food Chem. Toxicol.* **2002**, *40*, 1197–1203. [CrossRef]