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Original article

Enhancing effect of chia seeds on heterocyclic amine generation in meatball

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Summary The impact of using chia seeds at different rates (0%, 0.5%, 1%, and 1.5%) in meatball on the water content, pH and lipid oxidation (thiobarbituric acid reactive substances, TBARS), cooking loss and heterocyclic aromatic amines (HAAs) content of meatballs cooked at 150, 200, and 250 °C was investigated. The chia seed significantly affected water content ($P < 0.01$), cooking loss ($P < 0.05$), pH ($P < 0.05$) and total HAAs content ($P < 0.01$), whereas no significant impact ($P > 0.05$) was detected on TBARS value. With cooking, water content decreased ($P < 0.01$), while the pH and TBARS values increased ($P < 0.01$). Cooking temperatures significantly affected ($P < 0.01$) the water content, cooking loss and total HAAs content. The content of total HAAs of the meatballs increased with increasing the cooking temperature. The results illustrated that IQ, 7,8-DiMeIQx, 4,8-DiMeIQx, AαC and MeAαC were below the detectable limit (<LOD) in all samples, while MeIQx was the dominant compound among the detected HAAs compounds. The research indicated that the use of chia seeds increased the total HAA content, and the highest level was found in meatballs prepared with 1% chia seeds.

Keywords Chia seeds, heterocyclic aromatic amines, lipid oxidation, meatball, prooxidant.

Introduction

In order to maintain a healthy lifestyle food needs to be consumed, which has adequate nutritional and functional ingredients (Grdeń & Sołowiej, 2022; Wang *et al*., 2022). In this context, meat has an important place among the foods of animal origin in terms of nutrition due to its protein, fat, mineral and vitamin content (Libera *et al*., 2021). Meat is a complete protein source and eliminates deficiencies in basic foods taken into the human body in terms of amino acid composition. The human body more easily absorbs the iron in meat (heme iron) compared to the iron found in plant-based food (non-heme iron). Additionally, meat consumption can also enhance the absorption of the iron from other foods (non-heme form). Meat is also a rich source of B-group vitamins, with a higher bioavailability feature compared to plant-based foodstuffs (Bender, 1992; Karwowska *et al*., 2021).

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In consequence of cooking applied to meat and meat products (excluding products that can be consumed raw), the proteins are denatured and the meat becomes soft and digestible as a result of the partial hydrolysis of collagen (Song *et al*., 2021). Proper cooking procedures ensure the product's microbial safety and prolong its shelf life. Cooked meat flavour results from a number of reactions, including changes in lipids, carbohydrates and protein, thermal breakdown of peptides and amino acids, and reactions between proteins and carbohydrates (Bender, 1992; Van Boekel *et al*., 2010; Zamora & Hidalgo, 2019). While heat treatment causes water, mineral and vitamin losses in meat, there is a proportional increase in the amount of protein and fat due to water loss (Bender, 1992; Van Boekel *et al*., 2010; Aşçıoğlu, 2013; Zamora & Hidalgo, 2019). Heterocyclic aromatic amines (HAAs) can form as a consequence of certain reactions throughout the cooking of proteinaceous foods such as red meat and chicken (Sugimura, 2002). The

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formation of HAAs involves several complex reactions using the reducing sugars, amino acids and creati(ni)ne that are found naturally in meat. Maillard reaction, Strecker degradation and thermal pyrolysis of amino acids in meat are considered as important pathways in the formation of these compounds during the cooking process (Kizil *et al*., 2011; Gibis, 2016).

Positive relationships between HAAs and some types of cancer (pancreas, colorectum, breast, prostate) and between HAAs and mutagenicity have been determined in epidemiological studies (Sugimura, 1995; Skog *et al*., 1998; Klassen *et al*., 2002; Szterk & Jesionkowska, 2015; Zamora & Hidalgo, 2019; Meurillon *et al*., 2020). Therefore, HAAs are regarded as compounds that are responsible for the mutagenicity and carcinogenicity in meat (Lu *et al*., 2017). In fact, the International Cancer Research Center (IARC) evaluates IQ compound as ‘probable human carcinogen’ in class 2A and some of HAAs as ‘possible human carcinogens’ in class 2B (IARC, 1993).

In recent years, several studies have been conducted to estimate the HAAs content in various foodstuffs and the reduction effect of many substances’ usage on the amount of formed HAAs (OZ, 2011; Oz *et al*., 2015; Oz *et al*., 2021; Bingol *et al*., 2022; Fencioglu *et al*., 2022; Savaş *et al*., 2023). Two main reasons for this scientific interest exist. These are: (i) Determination that these compounds occur widely in cooked meat and meat products, (ii) Widespread consumption of meat and meat products that can contain these compounds in many countries. Therefore, most of the recent studies on HAAs have focused on reducing these compounds. In this context, since it is understood that oxidation reactions significantly contribute to the formation of HAAs, the use of antioxidant substances in manufacturing of meat products or the cooking of these products is considered the most practical way. The need for natural antioxidant substances derived from plant has gradually increased due to health problems caused by synthetic antioxidant substances. Plant-based antioxidant substances have a prooxidant influence relying on various factors such as the usage rate, environment and cooking conditions and consequently increase the formation of HAAs. For this reason, research has focused on the influence of matters with antioxidant activity on the HAAs formation.

Chia (*Salvia hispanica L.*) has been studied intensively recently because of its high nutrient content and functional characteristics (Ferreira *et al*., 2015; Câmara *et al*., 2020; Han *et al*., 2022). Chia seeds contain ~25–41% carbohydrates, 30–35% fat, 20–22% protein, 18–30% crude fibre (mainly indigestible cellulose) and 4–6% mineral substances (Bodoira *et al*., 2017; Zettel & Hitzmann, 2018). Chia is a valuable source of essential fatty acids (n-3 and n-6) and protein of high biological value (Romankiewicz *et al*., 2017; Yañez-Yazlle *et al*., 2021). As chia seeds contain essential amino acids especially valine, isoleucine, leucine and lysine at high levels, they can be complementary to cereal proteins, which are normally deficient in essential amino acids and can be used in the production of such food items including cookies, pasta and especially bread (Punia Bangar *et al*., 2022). In addition, chia seeds have the effect of increasing water-holding capacity (WHC) and viscosity because of their high dietary fibre content. In addition, chia seeds are a rich natural source of antioxidants in terms of phenolic compounds including tocopherols, sterols, carotenoids, chlorogenic and caffeic acid, myricetin, and kaempferol (Ferreira *et al*., 2015; Orona-Tamayo *et al*., 2015; Ding *et al*., 2018; Schettino-Bermúdez *et al*., 2020). Moreover, chia seeds contain high levels magnesium and have a significant amount of calcium and potassium content, which may be beneficial in controlling high blood pressure (Enes *et al*., 2020). Several studies have also reported that it has positive impact on health because of its various activities such as antioxidant, hypoglycaemic, hypolipidaemic and anti-inflammatory (Ghafoor *et al*., 2020; Oliva *et al*., 2020). Therefore, many studies in the literature examined the potential of using chia seeds as a fat replacer in meat products along with its ability to improve the products’ nutritional, sensory and quality characteristics. For example, chia seeds were used as an ingredient in various meat products such as camel burger (Zaki, 2018), chicken burgers (Paula *et al*., 2019) and fish burgers (Riernersman *et al*., 2016) in order to provide nutritional benefits and improve their texture and sensory properties. Even though many studies (Unal *et al*., 2018; Nuray & Oz, 2019; Elbir & Oz, 2021; Zhang *et al*., 2023) have been conducted examining the effects of different herbs and spices on HAAs, as far as we know, there has been no study examining the effect of the use of chia seed, which is considered a good antioxidant source, on HAAs formation. Thus, this is an important gap remains to be investigated in the literature. Therefore, the aim of the current research was to fill this gap by investigating the effect of chia seed usage, which has many advantages mentioned above, on HAAs formation and various quality criteria of the meatballs. For this purpose, meatballs produced by adding different levels of chia seeds (0, 0.5, 1, and 1.5, w/w) were analysed in terms of various quality criteria (water content, pH, lipid oxidation and cooking loss) and HAAs formation after cooking at different temperatures.

Materials and methods

Material

Raw materials

Beef meat (*M. Gluteus medius*) and intermuscular fat (to adjust the fat ratio of meatballs) used in the production of meatballs were purchased from the meat combine of Erzurum Meat and Milk Institution, Erzurum, Türkiye.

The meat was ground into minced meat and used for preparing the dough of meatball. The chia seeds added during the preparation of the meatball dough were bought from a local market in Erzurum, Türkiye.

Chemicals

Chemicals and/or solvents (including acetonitrile, methanol, ethyl acetate, sodium hydroxide, trichloroacetic acid) used for the analyses were either HPLC (High Performance Liquid Chromatography)-grade or analytical grade and purchased from Sigma-Aldrich, Germany. The HAAs standards were purchased from Toronto Research Chemicals (Toronto, Canada). Detailed information about the HAAs standards was given in Data S1.

Method

Meatball preparation and cooking

In the meatball preparation, firstly, the fat contents of both the beef meat (*M. Gluteus medius*) and intermuscular fat were determined according to the modified Babcock method. Thereafter, the minced meat was mixed with an appropriate amount of intermuscular fat, which was determined in Pearson's square method get meatball dough with 15% fat. Then, 15% fatty meatballs were divided into four pieces and chia seeds were not added to a randomly selected group that was evaluated as control group. Chia seeds powdered with a kitchen mixer were added to the other groups, at the rates of 0.5%, 1%, and 1.5% by weight. No any additional ingredients and/or spices were added during the meatball production. The meatball dough was left at 4 °C for 6 h, thereafter the meatballs were shaped using a ready-made steel globe mould with dimension of 7 × 1 cm to ensure that the meatballs are uniform in size and shape.

Meatball cooking was carried out on a hot plate. Cooking temperatures used in the present study were 150, 200, or 250 °C. A thermometer (Testo) was used for checking the temperature and the cooking time with a laboratory stopwatch. A total of 8 min (4 min for each side) cooking time was applied to the meatballs. For HAAs analyses, the homogenised samples were stored at −18 °C, thawed in refrigerator conditions the day before the analysis and used in the analysis.

Analyses

Water content. The water content of the meatballs was determined according to Gökalp *et al.* (2010). Detailed information about the analysis was given in Data S1.

pH value. The pH analysis of the meatballs was determined according to Gökalp *et al.* (2010). Detailed information about the analysis was given in Data S1.

Lipid oxidation (thiobarbituric acid reactive substances). The lipid oxidation level (thiobarbituric acid reactive substances [TBARS]) of the meatballs was determined according to Kılıc & Richards (2003). Detailed information about the analysis was given in Data S1.

Cooking loss. The percentage of cooking losses of the meatballs was determined according to Oz & Kızıl (2013). Detailed information about the analysis was given in Data S1.

HAAs analysis. The HAAs analysis of the meatballs was determined according to Oz *et al.* (2016). Detailed information about the analysis was given in Data S1.

Statistical analysis. The current study was set up using the Randomised Complete Block Design method, with two repetitions being performed. The data were analysed using the SPSS software package and Duncan's multiple comparison test was applied to identify significant differences between mean values that were found to be statistically significant. The SIMCA 14.1 program (UMETRICS, Umea, Sweden) was employed to conduct Principal Component Analysis (PCA).

Results and discussion

The water content, pH and TBARS values of beef meat, beef fat and raw meatballs

Table 1 gives the results of water content, pH and TBARS analyses of beef meat (*M. Gluteus medius*) and meat fat (intermuscular fat) belonging to the same carcass used to adjust the fat content of meatballs used as materials in the study and raw.

The beef meat used in this study had a water content of 74.11%, a pH of 5.80 and a TBARS value of 0.180 mg MDA/kg. The results obtained are generally in line with those documented in previous researches. Previous studies where beef meat was used as a raw

TABLE 1 The water content, pH and TBARS value of beef meat and intermuscular fat used for the preparation of the meatballs and raw meatball (with 15% fat)

Sample	*n*	Water (%)	pH	TBARS (mg MDA/kg)
Meat	2	74.11 ± 0.13^{a}	5.80 ± 0.08^{b}	0.180 ± 0.071^{a}
Intermuscular fat	2	22.98 ± 2.81^{c}	7.53 ± 0.05^{a}	0.140 ± 0.014^{a}
Meatball	2	66.40 ± 2.05^{b}	5.90 ± 0.02^{b}	0.230 ± 0.028^{a}
Significance		**	**	ns

ns, $P > 0.05$; **, $P < 0.01$.

[a,b,c]Mean values with different letters are significantly different from each other.

TBARS, thiobarbituric acid reactive substances.

material, Oz *et al.* (2017) found the water content, pH and TBARS values as 72.29%, 5.55 and 0.41 mg MDA/kg, respectively, Oz & Zikirov (2015) found the water and pH values as 72.54% and 5.56%, respectively, and Oz & Çakmak (2016) determined the water and pH values as 62.16% and 5.51%, respectively. In their study, Korkmaz & Oz (2020) determined the water content of beef meat as 74.02%, its pH value as 5.59, and its TBARS value as 0.872 mg MDA/kg, while these values were determined by Bulan & Oz (2022) as 74.11%, 5.80 and 0.82 mg MDA/kg, respectively.

The water content of intermuscular meat fat used during meatballs preparation was determined as 22.98%, whereas its pH value was found to be 7.53, and its TBARS value to be 0.140 mg MDA/kg. In their study, Korkmaz & Oz (2020) determined the water content of the intermuscular meat fat, which was used to adjust the fat ratio of meatballs to 15%, as 13.34, the pH value as 6.16 and the TBARS value as 0.255 mg MDA/kg. Ekiz (2017) determined the water content of intermuscular fat, which was used in the production of beef meatballs as 9%, whereas the pH value was reported as 6.57 and the TBARS value as 0.22 mg MDA/kg, while these values were found by Uzun & Oz (2021) to be 19.78%, 6.77 and 0.251 mg MDA/kg, respectively.

Regarding the raw meatballs with 15% fat prepared in this study, the water content was found to be 66.40%, whereas their pH value was 5.90, and the TBARS value was 0.230 mg MDA/kg. Korkmaz & Oz (2020) determined the water content of raw meatballs with 15% fat as 67.21%, pH value as 5.71 and TBARS value as 0.656 mg MDA/kg. These values were reported as 63.85%, 5.75 and 0.63 mg MDA/kg by Zaman (2014), as 65.66%, 5.46 and 1.29 mg MDA/kg by Ekiz (2017) and as 61.75%, 5.70 and 0.215 mg MDA/kg by Bingol *et al.* (2022), respectively.

Water content

The water contents of raw and cooked meatballs without and with chia seeds are presented in Table 2.

It was seen that the use of chia seeds at different rates, the cooking status and the cooking temperature had a significant impact ($P < 0.01$) on the water content of the meatballs. Whereas the highest water content was determined in the meatball samples with 0.5% chia seeds, the water contents of the other samples did not vary significantly. In the literature, it is declared that the chia seed has diverse effects on the water content of meat products. Yüncü *et al.* (2022) stated that meatball samples produced with chia mucilage had higher water content in comparison to control group, while Antonini *et al.* (2020) found that water content of burgers with chia seeds significantly decreased. In another study, Paula *et al.* (2019) found that water activity values of chicken burgers produced with chia seeds at different rates (2%, 4%, and 8%) were similar to water activity values of the control group. After cooking process, the water content of the meatballs, which was 65.55% (n = 24) on average before cooking, significantly ($P < 0.01$) decreased, as expected, to 56.44%. This decrease is considered to be related to the shrinkage of myofibrillar proteins and perimysial connective tissue that occurs during cooking (Sánchez del Pulgar *et al.*, 2012). In addition, it was

TABLE 2 The effects of chia usage rate, cooking process and cooking temperature on the water content, pH, TBARS and cooking loss value of the meatballs

	n	Water (%)	pH	TBARS (mg MDA/kg)	Cooking loss (%)
Chia usage rate (%)					
0	12	60.74 ± 4.46^{b}	6.02 ± 0.18^{a}	0.624 ± 0.082^{a}	$32.60 \pm 1.83^{a,b}$
0.5	12	62.07 ± 4.87^{a}	5.98 ± 0.20^{b}	0.612 ± 0.122^{a}	30.79 ± 4.62^{b}
1	12	60.94 ± 5.47^{b}	6.03 ± 0.15^{a}	0.657 ± 0.046^{a}	34.10 ± 3.49^{a}
1.5	12	60.23 ± 4.95^{b}	6.02 ± 0.15^{a}	0.617 ± 0.066^{a}	33.18 ± 2.00^{a}
Significance		**	*	ns	*
Cooking process					
Raw	24	65.55 ± 1.06^{a}	5.86 ± 0.05^{b}	0.582 ± 0.059^{b}	-
Cooked	24	56.44 ± 1.84^{b}	6.17 ± 0.04^{a}	0.672 ± 0.080^{a}	-
Significance		**	**	**	-
Cooking temperature (°C)					
150	16	61.74 ± 4.29^{a}	6.00 ± 0.16^{a}	0.626 ± 0.108^{a}	30.25 ± 3.78^{b}
200	16	60.46 ± 5.37^{b}	6.02 ± 0.18^{a}	0.616 ± 0.056^{a}	34.58 ± 1.97^{a}
250	16	60.79 ± 5.02^{b}	6.02 ± 0.17^{a}	0.640 ± 0.081^{a}	33.18 ± 2.20^{a}
Significance		**	ns	ns	**

ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$.

^{a,b}Mean values with different letters are significantly different from each other.

TBARS, thiobarbituric acid reactive substances.

determined that increasing from 150 to 200 °C decreased ($P < 0.01$) the water content, but the water contents of the meatballs cooked at 200 °C did not show a significant difference ($P > 0.05$) compared to those cooked at 250 °C. There are studies with similar findings in the literature (Oz & Çakmak, 2016; Oz *et al.*, 2016; Bingol *et al.*, 2022; Bulan & Oz, 2022).

pH value

The pH values of raw and cooked meatballs without and with chia seeds are also presented in Table 2. The use of chia seeds at different rates showed a significant effect ($P < 0.05$) on the samples' pH values, and the cooking process also showed a very significant effect ($P < 0.01$), whereas the cooking temperature did not have a significant effect ($P > 0.05$) on their pH values. While the lowest pH value was determined in the meatballs with 0.5% chia seeds, it was seen that the pH values of the remaining samples did not vary significantly from each other. In the literature, it is stated that the use of chia seeds in meat products has diverse effects on the pH value. Indeed, Antonini *et al.* (2020) reported that while their study indicated that beef burger samples prepared with chia seeds had lower pH values in comparison with the control group, the rate of chia seed use had no significant effect on pH values. Another study reported that pH values of chicken meat burgers prepared by adding chia seeds at different rates (0%, 2%, 4%, and 8%) varied between 6.2 and 6.3, and the difference between pH values was statistically insignificant (Paula *et al.*, 2019). Similarly, the use of chia seeds at different rates (0%, 1%, 3%, and 5%) in fresh burger samples prepared from camel meat was shown to have no effect on pH values (Zaki, 2018). Because of the cooking process, the meatballs' pH value, which was 5.86 (n = 24) on average before cooking, significantly ($P < 0.01$) increased, as expected, to 6.17. This increase is considered to be related to the release of bonds containing sulfhydryl, imidazole and hydroxyl groups with the cooking (Girard, 1992). A similar increment in meatballs' pH values due to the application of heat treatment was also documented by Uzun & Oz (2021) and Bingol *et al.* (2022). On the other hand, as in the current research, Oz *et al.* (2016), Lu *et al.* (2018), Korkmaz & Oz (2020) and Bingol *et al.* (2022) also reported that cooking temperatures applied during cooking process did not affect pH values of meatball samples.

TBARS values

The TBARS values of raw and cooked meatballs without and with chia seeds are also presented in Table 2. While the use of chia seeds at different rates and the cooking temperature did not significantly affect ($P > 0.05$) the meatballs' TBARS values, the cooking process increased the TBARS value significantly ($P < 0.01$). The TBARS values of the meatballs produced by adding chia seeds at different rates varied between 0.612–0.657 mg MDA/kg and the TBARS values of the meatballs were not statistically different from each other ($P > 0.05$). In our study, it was expected that chia seeds used in meatball production would show a reducing or inhibiting effect on lipid oxidation of meatball samples owing to the high polyphenol content and antioxidant activity of chia seeds. However, no such effect of chia seeds was observed in current research. It is thought that the high content of polyunsaturated fatty acids in chia seeds may affect this situation. On the other hand, it is declared in the literature that the chia seeds may have different effects on lipid oxidation in meat products. Indeed, Antonini *et al.* (2020) mentioned that use of chia seeds in beef burger production provided a significant reduction in malondialdehyde level, and the observed decrease was not dose-dependent. Similarly, Paula *et al.* (2019) declared that TBARS values of chicken burger samples prepared with chia seeds were lower than that of control group samples, and TBARS values decreased with increase in chia seed ratio. On the other hand, Frankfurter sausage samples prepared with chia flour were also reported to show a higher TBARS value compared to that of control samples (Pintado *et al.*, 2016). The TBARS value (n = 24) of the meatballs, which was 0.582 mg MDA/kg on average before cooking, increased significantly ($P < 0.01$), as expected, after the cooking process and increased to 0.672 mg MDA/kg. It is evaluated that this increase is related to the release of iron in meat proteins during the cooking process and the damage to the meat's cellular structure by the cooking process (Ramírez *et al.*, 2005; Rojas & Brewer, 2007). On the other hand, as in the current research, Oz *et al.* (2016), Kilic *et al.* (2021), Uzun & Oz (2021) and Bulan & Oz (2022) also reported that the cooking temperatures applied during the cooking process had no effect on the TBARS values of the meatball samples.

Cooking loss

The cooking loss percentages of the meatballs produced by adding chia seeds at various rates and cooked at several temperature levels are also presented in Table 2. The use of chia seeds at different rates had a significant ($P < 0.05$) effect on the cooking loss values of the samples, while the cooking temperature had a very significant ($P < 0.01$) effect. The cooking loss values of the meatball samples with 0.5% chia seeds were lower than that of the meatballs prepared with 1% and 1.5% chia seeds. This finding is also consistent with the water content of the samples mentioned. As a matter of fact, the water content of the meatballs prepared with 0.5% chia seeds was found to be higher than those of meatballs prepare with 1% and 1.5% chia seeds. Antonini *et al.* (2020) reported that burger samples prepared by adding chia seeds showed a lower cooking loss value than that of control group samples, and a reduction in cooking loss values was observed as chia seed ratio increased. On the other hand, Paula *et al.* (2019) reported no statistically significant difference between cooking loss values of chicken burgers produced with different amounts of chia seeds. In the present study, it was found that when the cooking temperature rose from 150 to 200 °C led to a very significant ($P < 0.01$) increase in the samples' cooking loss values, but, no significant ($P > 0.05$) differences were detected in those cooked at 200 and 250 °C. There are similar studies in the literature in which the cooking loss values of meatballs change depending on the different cooking temperatures (Oz & Çakmak, 2016; Oz *et al.*, 2016; Tengilimoglu-Metin *et al.*, 2017; Oz, 2019; Korkmaz & Oz, 2020; Uzun & Oz, 2021; Bingol *et al.*, 2022).

Table 3 The recoveries, the LOD and the LOQ values of the HAAs analysed

HAA	Recovery (%)	LOD (ng/g)	LOQ (ng/g)
IQx	94.44	0.004	0.013
IQ	82.17	0.009	0.029
MeIQx	93.07	0.024	0.081
MeIQ	81.52	0.014	0.047
7,8-DiMeIQx	92.96	0.005	0.018
4,8-DiMeIQx	94.56	0.008	0.025
PhIP	95.44	0.025	0.085
AαC	95.64	0.012	0.039
MeAαC	90.53	0.010	0.035

Abbreviations: HAA, heterocyclic aromatic amine; LOD, limit of detection; LOQ, limit of quantification.

Recoveries of the HAAs

The recoveries of the HAAs compounds investigated in this study were calculated according to the standard addition technique by adding the analytes at known concentrations. The recoveries of HAAs, and limit of detection (LOD) and limit of quantification (LOQ) values, are presented in Table 3. The recovery values of HAAs varied between 81.52–95.64%, the LOD values (detection limit = 3) and the LOQ values (measurement limit = 10) varied between 0.004–0.025 and 0.013–0.085 ng/g, respectively. Current recoveries and the LOD and LOQ values are in line with the literature (Messner & Murkovic, 2004).

HAAs results

In the current study, nine different HAAs compounds were examined (Table 4). While the analysed samples did not contain any IQ, 7,8-DiMeIQx, 4,8-DiMeIQx, AαC and MeAαC compounds at detectable levels, varying levels of IQx, MeIQ and PhIP compounds were determined. However, it was found that the most commonly found compound in the study was MeIQx, and the compound detected in the highest amount was PhIP. Similar results were found by Uzun & Oz (2021) and Bingol *et al.* (2022) who reported MeIQx as the most frequently determined HAAs compound in meatballs produced with astaxanthin and basil, respectively, that were cooked at temperatures ranging from 150 to 250 °C. They also reported IQ, AαC and MeAαC compounds at levels below the LOD in the cooked samples. The absence of AαC and MeAαC, which are

TABLE 4 The HAA amounts of the cooked meatballs without and with chia seed (ng/g)

Cooking temperature (°C)	Chia usage rate (%)	IQx	IQ	MeIQx	MeIQ	4,8-DiMeIQx	7,8-DiMeIQx	PhIP	AαC	MeAαC	Total HAA
150	0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	0.5	nd	nd	nq	nd	nd	nd	nd	nd	nd	nd
	1	nd	nd	0.12 ± 0.01	nd	nd	nd	nd	nd	nd	0.12 ± 0.01
	1.5	nd	nd	0.08 ± 0.00	nd	nd	nd	nd	nd	nd	0.04 ± 0.00
200	0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	0.5	nd	nd	0.12 ± 0.04	nd	nd	nd	nd	nd	nd	0.12 ± 0.04
	1	nd	nd	0.30 ± 0.09	nd	nd	nd	nd	nd	nd	0.30 ± 0.09
	1.5	nd	nd	0.24 ± 0.04	nq	nd	nd	nd	nd	nd	0.24 ± 0.04
250	0	nd	nd	nq	nd	nd	nd	nd	nd	nd	nd
	0.5	0.02 ± 0.01	nd	0.22 ± 0.05	nq	nd	nd	nd	nd	nd	0.24 ± 0.06
	1	0.06 ± 0.03	nd	0.31 ± 0.19	0.18 ± 0.03	nd	nd	0.54 ± 0.15	nd	nd	1.08 ± 0.34
	1.5	0.05 ± 0.04	nd	0.33 ± 0.03	0.14 ± 0.07	nd	nd	nq	nd	nd	0.52 ± 0.14

Note: nd < LOD; nq < LOQ.
Abbreviations: HAA, heterocyclic aromatic amine; LOD, limit of detection; LOQ, limit of quantification.

pyrolytic HAA compounds, is thought to be because they form by the hydrolysis of amino acids such as tryptophan, phenylalanine, etc. at very high temperatures (above 250 °C). In this context, the fact that these compounds could not be detected even at the highest temperature (250 °C) in the present study is thought to be due to the fact that they occur at very high temperatures, as well as the cooking method and time (Meurillon & Engel, 2016). Because it is stated that these compounds are mostly formed in cooking methods such as barbecue (Oz & Kaya, 2011).

As can be seen from Table 4, the levels of HAAs in the control group meatballs were below their LOD values, except for MeIQx that was found at an amount less than its LOQ value in the samples cooked at a high temperature (250 °C). On the other hand, all meatballs produced with chia seed had MeIQx levels higher than the LOQ, except for samples that were cooked at a low temperature (150 °C). IQx was able to be found at levels higher than its LOQ value only in the meatballs with chia seeds and cooked at a high temperature (250 °C). MeIQ was able to detect in the meatballs with chia seeds and cooked at a high temperature (250 °C). Furthermore, the sample with 1% chia seeds cooked at 250 °C was the only sample that contained PhIP at levels higher than the LOQ. In addition, interestingly adding chia seeds at a rate of 1% resulted in higher amounts of IQx, MeIQ and PhIP compounds compared to the other rates (0.5% and 1.5%). Thus, it could be concluded that the use of chia seeds enhanced the formation of some HAA compounds (IQx, MeIQ and PhIP) in meatballs, especially when a high cooking temperature was applied. Various studies on different types of meats reported the enhancement effect of several plants, herbs and spices on the formation of HAA compounds depending on the used concentration and cooking temperature (Uzun & Oz, 2021; Bingol *et al.*, 2022; Bulan & Oz, 2022). In contrast, the reduction effect of phenolic compounds of various spices and herbs extracts and including garlic, onion, paprika, ginger, pepper, rosemary extract, turmeric and cumin on the levels of different HAA compounds was determined (Puangsombat & Smith, 2010; Puangsombat *et al.*, 2011; Lu *et al.*, 2018).

A lot of factors such as meat type, precursors present in the meat, cooking conditions, extraction method, analysis equipment etc. affect on HAA formation. Therefore, it is very difficult to compare the results obtained in the current study with the data in the literature. However, when a general evaluation is made, it can be stated that the results obtained in the current study are lower than the data in the literature. It is thought that the differences between them are due to the mentioned factors. Indeed, Shan *et al.* (2021) developed a novel magnetic solid-phase extraction method for detection of HAAs and investigated HAAs content in commercial meat products. The researchers determined 3.06 ng/g IQx, 0.43 ng/g IQ, 33.51 ng/g MeIQx, 1.82 ng/g MeIQ, 1.36 ng/g 7,8-DiMeIQx, 3.74 ng/g PhIP and 3.09 ng/g AαC in commercial beef products, while MeAαC could not be detected. Zhang *et al.* (2021) determined 6.50 ng/g IQ, 0.51 ng/g MeIQx, 4.47 ng/g MeIQ, 3.96 ng/g 4,8-DiMeIQx, 1.52 ng/g 7,8-DiMeIQx and 7.61 ng/g PhIP in fried steak, while IQx could not be detected.

The concentration of total HAAs in cooked meatballs with and without chia seeds are given in Table 5 in a statistically comparative approach. It was noticed that both the cooking temperature and the concentration of chia seed exhibited a statistically significant effect ($P < 0.01$) on the meatballs' total HAAs content.

While the total HAAs content in the control group remained below the detectable level (LOD), the total HAAs content in the meatballs containing chia seeds at different rates was found to vary between 0.12–0.50 ng/g. The highest total HAAs content was determined in the meatballs with 1% chia seeds. In this study, the use of chia seeds in the preparation of meatballs caused the formation of some individual HAAs that were lower than the LOD values in the control group, resulting in an increase in the total HAAs content. The basic mechanism behind the enhancing effect of chia seed on the HAAs content is not fully known. However, it is thought to be due to the nutritional composition of chia seeds, which may be the determining factor in this effect. This is due to the fact that the high carbohydrate content of chia seeds as well as the high protein; and therefore, amino acid content can provide the necessary substrates (reducing sugar and free amino group) for the Maillard reaction to occur. Because it is known that chia seeds contain ~20–22% protein and 25–41% carbohydrates (Martínez *et al.*, 2012; Marineli *et al.*, 2014; Bodoira *et al.*, 2017; Zettel & Hitzmann, 2018; Punia &

TABLE 5 The effects of chia usage rate and cooking temperature on the total HAA content of the meatballs (ng/g)

	n	Total HAA
Chia usage rate (%)		
0	2	nd[c]
0.5	2	0.12 ± 0.11[c]
1	2	0.50 ± 0.49[a]
1.5	2	0.28 ± 0.21[b]
Significance		**
Cooking temperature (°C)		
150	8	0.05 ± 0.05[b]
200	8	0.16 ± 0.13[b]
250	8	0.46 ± 0.45[a]
Significance		**

**, $P < 0.01$.

HAA, heterocyclic aromatic amine.

	Water Content	pH	TBARS	Cooking Loss	Total HAA
Water Content	1				
pH	-0.29	1			
TBARS	0.57	0.39	1		
Cooking Loss	-0.83**	0.01	-0.60*	1	
Total HAA	-0.22	0.04	0.25	0.35	1

FIGURE 1 Correlation analysis of the meatballs.

Dhull, 2019). In addition, it has been reported that some compounds (such as phenolic compounds, flavonols and isoflavones) possessed by chia seeds add antioxidant properties to chia seeds (Ferreira *et al.*, 2015; Orona-Tamayo *et al.*, 2015; Gómez-Favela *et al.*, 2017; Ding *et al.*, 2018; Schettino-Bermúdez *et al.*, 2020; Knez Hrnčič *et al.*, 2020). On the other hand, it has been noted in previous studies that antioxidant substances present in the cooking media can have a prooxidant effect and promote the formation of HAAs depending on ambient conditions, cooking conditions, concentrations, structures and substrates present in the environment (Fencioglu *et al.*, 2022). In the current study, it is believed that some compounds with antioxidant activity in the chia seed increase the formation of HAAs by showing a prooxidant effect. In this context, it was also observed that the use of chia seed in meatball production did not show a reducing effect on the TBARS values of the samples (Table 2). On the other hand, studies have reported that chia seeds are a potential source of antioxidants containing chlorogenic acid, caffeic acid, myricetin, quercetin and kaempferol, which are believed to have heart, liver protective, anti-ageing and anti-cancer properties. Chia seeds also contain caffeic acid, chlorogenic acid and quercetin, which have been linked to high phenolic levels (Ullah *et al.*, 2016). Another issue that should be emphasised here is the chlorogenic acid found in chia seeds. In addition to its antioxidant properties, chlorogenic acid is also known to have antibacterial, anticarcinogenic, hypoglycaemic and hypolipidaemic effects (Stalmach *et al.*, 2006; Bassoli *et al.*, 2008). It is also stated that as a result of heat treatment, chlorogenic acid is converted to lactones of caffeoyl, coumaroyl- or feruloylquinic acids and various aromatic compounds are formed from carbohydrates, proteins, fats and aromatic acids during pyrolysis (Gruenwald *et al.*, 2000; Farah *et al.*, 2005; Poisson *et al.*, 2009).

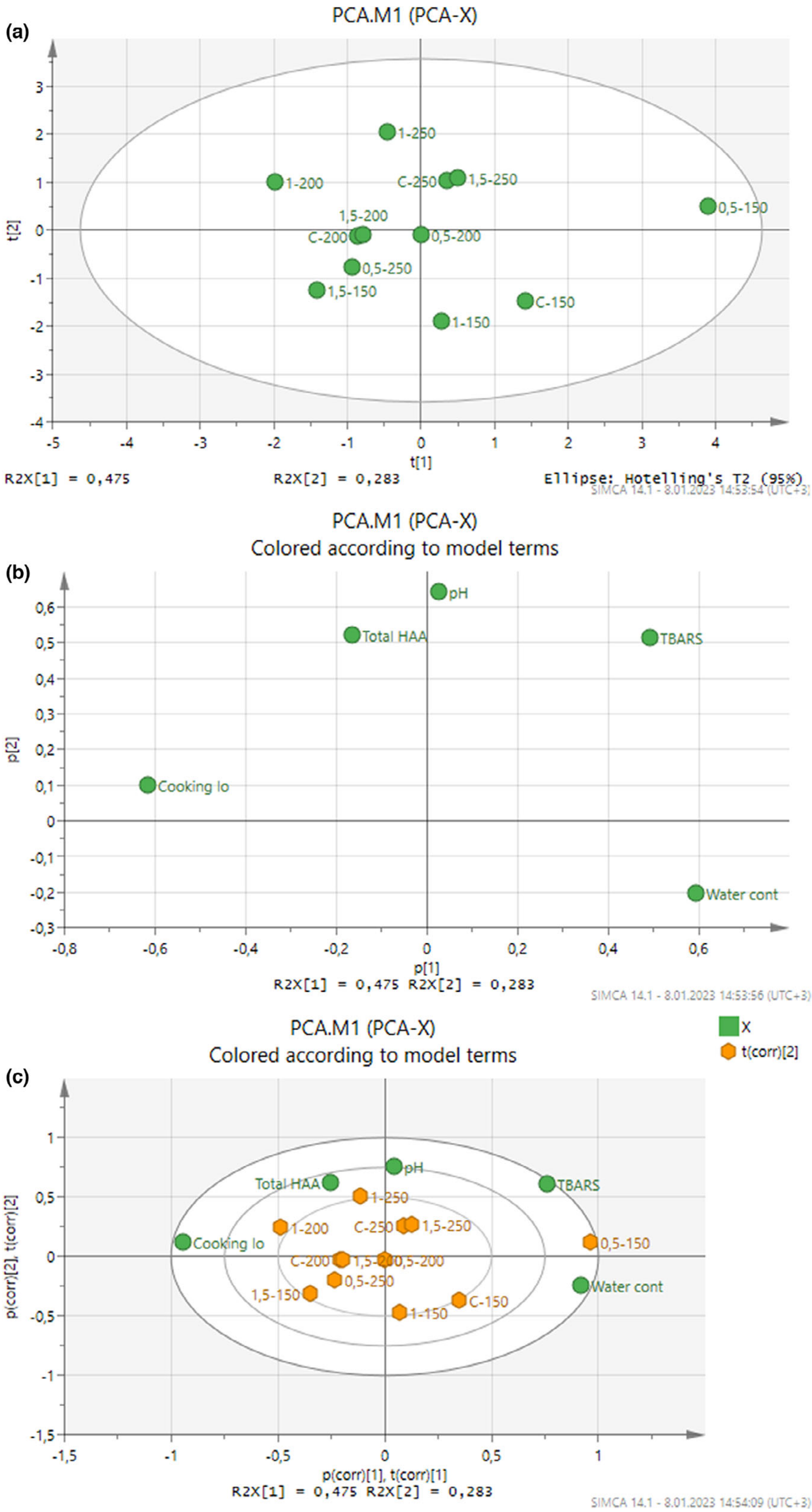
This study illustrated that the increase in cooking temperature caused a raise in the total HAAs content. On the other hand, it was determined that this increase in the total HAAs content did not have a significant effect on the increase of the cooking temperature from 150 to 200 °C, but only had a significant effect on the increase from 200 to 250 °C. When evaluated in terms of cooking temperature, it was revealed that the total HAAs amount varied from 0.05 to 0.46 ng/g. In parallel with the current research, Teng *et al.* (2023) also reported that there is a positive correlation between the cooking temperature and the formation of HAAs and that the HAAs content increased as the cooking temperature increased. Similar results were obtained in some studies in the literature and total HAAs content was determined at varying levels (Tsen *et al.*, 2006; Tengilimoglu-Metin *et al.*, 2017). Nor Hasyimah *et al.* (2020) stated that total HAAs content increased as temperature increased in honey-marinated beef samples cooked at various grill temperatures (150, 250, and 350 °C), and total HAAs values of control group samples varied between 27.34 and 629.53 ng/g.

It is seen that the results of the present investigation and those found in the literature are quite comparable. It is thought that the differences in the results of our study and the studies in the literature are due to factors such as raw materials and additives used in meatball production, cooking conditions (method, temperature, time, and equipment), analysis methods, etc. (Kilic *et al.*, 2021).

Correlation analysis and samples' discrimination by PCA

Pearson correlation between the water content, pH, TBARS, cooking loss and total HAAs content of the meatballs is given in Fig. 1. An expected strong

FIGURE 2 Score scatter plot (a), loading scatter plot (b), and biplot (c) of PCA analysis (PC1 vs. PC2) for the components in the meatballs. C-150: The control group meatballs cooked at 150 °C; C-200: The control group meatballs cooked at 200 °C; C-250: The control group meatballs cooked at 250 °C; 0.5–150: The meatballs with 0.5% chia and cooked at 150 °C; 0.5–200: The meatballs with 0.5% chia and cooked at 200 °C; 0.5–250: The meatballs with 0.5% chia and cooked at 250 °C; 1–150: The meatballs with 1% chia and cooked at 150 °C; 1–200: The meatballs with 1% chia and cooked at 200 °C; 1–250: The meatballs with 1% chia and cooked at 250 °C; 1.5–150: The meatballs with 1.5% chia and cooked at 150 °C; 1.5–200: The meatballs with 1.5% chia and cooked at 200 °C; 1.5–250: The meatballs with 1.5% chia and cooked at 250 °C.


(a)
PCA.M1 (PCA-X)
1-250
1-200
C-250
1,5-250
0,5-150
1,5-200
C-200
0,5-200
0,5-250
1,5-150
C-150
1-150
t[2]
t[1]
R2X[1] = 0,475
R2X[2] = 0,283
Ellipse: Hotelling's T2 (95%)
SIMCA 14.1 - 8.01.2023 14:53:54 (UTC+3)
(b)
PCA.M1 (PCA-X)
Colored according to model terms
pH
Total HAA
TBARS
Cooking lo
Water cont
p[2]
p[1]
R2X[1] = 0,475 R2X[2] = 0,283
SIMCA 14.1 - 8.01.2023 14:53:56 (UTC+3)
(c)
PCA.M1 (PCA-X)
Colored according to model terms
X
t(corr)[2]
pH
Total HAA
TBARS
1-250
1-200
C-250
1,5-250
Cooking lo
0,5-150
C-200
1,5-200
0,5-200
0,5-250
1,5-150
Water cont
C-150
1-150
p(corr)[2], t(corr)[2]
p(corr)[1], t(corr)[1]
R2X[1] = 0,475 R2X[2] = 0,283
SIMCA 14.1 - 8.01.2023 14:54:09 (UTC+3)

negative correlation between the cooking loss and water content (r = −0.83, $P < 0.01$) was observed, indicating that water loss is the main contributor to the weight loss of meatball samples during the cooking process (loss in other components such as fat, minerals and vitamins could be neglected). A similar relation between the cooking loss and water content (r = −0.70, $P < 0.01$) was also reported by Kilic *et al.* (2021). Additionally, our results revealed a negative correlation between cooking loss and TBARS values (r = −0.60, $P < 0.05$). Similarly, Klinhom *et al.* (2017) also reported a negative correlation between cooking loss and TBARS values (r = −0.54, $P < 0.05$). This relationship could be attributed to flowing out the MDA (a by-product of lipid peroxidation determined by TBARS analysis) with the water released during the cooking process (expressed as cooking loss) since MDA is a water-soluble compound, that is increasing the cooking loss increases the exuded MDA and consequently, reducing the measured TBARS values (Klinhom *et al.*, 2017). No significant correlations were found between the total HAAs and other analyses (Fig. 1). In contrast, Savaş *et al.* (2021) observed a negative correlation between the total HAAs content and water content (r = −0.45, $P < 0.05$), pH (r = −0.80, $P < 0.01$), and cooking loss (r = −0.52, $P < 0.01$).

Principal Component Analysis was employed to visually distinguish the differences in the characteristics of meatballs with and without chia seeds, by assessing their water content, cooking loss, pH, TBARS value and total HAAs contents. The score scatterplot, loading scatter plot and biplot, are presented in Fig. 2a–c, respectively. The first two principal components (PC1 = 47.5% and PC2 = 28.3%) accounted for 75.8% of the variance. The samples with 0.5% chia seed and cooked at 150 °C were well separated from the other samples (Fig. 2a), suggesting that they had some differences. While cooking loss and total HAAs content were clustered together at the left side of the plot, water content, pH and TBARS were clustered together at the right side of the plot (Fig. 2b). Cooking loss was negatively correlated with water content and TBARS (Fig. 2b). As to total HAAs content, it is seen that the samples closest to the total HAAs content are the meatball samples cooked at 200 and 250 °C and containing 1% chia seeds, which means that the total HAA contents are higher in these samples compared to the other samples (Fig. 2c).

Conclusion

Adding different ratios (0%, 0.5%, 1%, and 1.5%) of chia seeds to meatballs that were cooked at various temperatures (150, 200, and 250 °C) significantly influenced their water content, cooking loss, pH value and content of total HAAs. Even though the high antioxidant activity of chia seeds have been reported in several studies, no significant differences were found between the samples produced with and without chia seeds in terms of their TBARS values. Meatballs produced with 0.5% chia seeds exhibited higher water content, lower cooking loss and lower pH value compared to other samples. Interestingly, the use of chia seeds in meatball production enhanced the formation of various HAAs compounds (IQx, MeIQx, MeIQ, and PhIP), especially when cooked at higher temperatures. The highest content of total HAAs (1.08 ng/g) was determined in the meatballs produced with 1% chia seed and cooked at 250 °C. Further study is required to evaluate the impact of wider usage rates and cooking temperatures on the quality properties of meatballs and the effect of chia seeds addition on the shelf life of meatballs.

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Author contributions

Zeynep Elbir: Formal analysis (equal); investigation (equal); writing – original draft (equal). **Elif Ekiz:** Formal analysis (equal); investigation (equal); writing – original draft (equal). **Eyad Aoudeh:** Formal analysis (equal); investigation (equal); writing – original draft (equal). **emel Oz:** Formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Adem Savas:** Formal analysis (equal). **Charles Stephen Brennan:** Investigation (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Charalampos Proestos:** Writing – original draft (equal); writing – review and editing (equal). **Mohammad Rizwan Khan:** Writing – original draft (equal); writing – review and editing (equal). **Tahra El Obeid:** Writing – original draft (equal); writing – review and editing (equal). **Margaret Brennan:** Writing – original draft (equal); writing – review and editing (equal). **Fatih ÖZ:** Conceptualization (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal).

Conflict of interest statement

The authors have declared no conflicts of interest.

Peer review

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Data availability statement

The data that support the findings of this study are available from the authors upon reasonable request.

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This article is important for our manuscript. Because the information about the chlorogenic acid was taken from this article.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. HAAs standards and analysis methods.