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Research Article

Effect of different wood species on heterocyclic aromatic amine level in Harbin red sausages

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Abstract: The influence of different wood species (in the form of wood chips) on the formation of heterocyclic aromatic amines (HAAs) in smoked Harbin red sausages was investigated. Four common species of wood (pear, oak, apple, beech) were used for smoking. The smoking process significantly affected the moisture content, water activity, pH, lipid oxidation (thiobarbituric acid-reactive substances), protein oxidation (carbonyl content) and HAA content. It was found that the wood species significantly influenced the contents of HAAs in the smoked samples. Total HAA contents were highest in samples smoked using wood chips produced from pear, followed by oak, beech and apple. The contents of Norharman and Harman were much higher than those of the other HAAs. Lipid oxidation and protein oxidation were significantly associated with the formation of total HAAs in samples. It is shown that the type of wood chips used for smoking is one of the critical parameters affecting the contamination of HAAs in smoked meat products.

Keywords: heterocyclic aromatic amine; wood chip; harbin red sausage; lipid oxidation; protein oxidation

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1 Introduction

Heterocyclic aromatic amines (HAAs) are a group of carcinogenic compounds that cause genotoxic damage by reacting with DNA[1–2]. Up to now, more than 30 HAAs have been separated and determined at nanogram per gram levels in cooked foods, mainly protein-rich foods, including meat, poultry and fish[3–4]. HAAs are also found in ashes generated after smoking, which could be exposed to the processing environment through atmospheric particulate material[5].

According to the molecular structures and formation pathways, HAAs can be divided into polar HAAs or aminoimidazoazerene HAAs, and non-polar HAAs or aminocarboline HAAs[1]. The polar HAAs are mainly composed of the imidazoquinoline, imidazoquinoxaline and imidazopyridine types, formed by the heat-induced Maillard reaction during conventional cooking temperatures between 150 and 300 ℃[6]. The non-polar HAAs are mainly composed of dipyridoimidazole and pyridoindole moieties, formed by the pyrolysis of amino acids at temperatures over 300 ℃[3].

Several factors affect the contents of HAAs produced in cooked meats, including the type of meat, cooking temperature, cooking time and cooking method, such as frying, grilling, boiling, roasting and smoking[7]. HAAs tend to form at temperatures > 150 ℃[3,8], but their detection in meat products smoked at relatively lower temperatures has also been demonstrated[9–10]. Therefore, it is important to understand the factors affecting the formation of HAAs in foods cooked at low temperatures (< 150 ℃) so that the risk of exposure can be minimised.

The smoke produced by the thermal combustion of wood is used for food preservation[11] and, currently, mainly for flavour enhancement. The pyrolysis of wood begins with water evaporation, followed by decomposition of the hemicelluloses, cellulose and, finally, lignin[12]. Generally, wood is approximately 50% cellulose, 25% hemicellulose and 25% lignin[12]. Therefore, the wood smoke composition mainly depends on the wood type and the heat-induced chemical reactions between the heated polymers, gasified intermediates and moisture, ultimately influencing smoked food quality and safety. Although some studies have investigated the HAAs formation in smoked meat products as a function of the smoking temperature, smoking method and smoking time[8,10], there is little information on the influence of different species of wood on HAAs formation. Therefore, it is of great significance to investigate the effects of the commonly used woods that can produce high-quality flavour on the safety (HAAs formation) of smoked meat products. Additionally, the pathways involved in the formation of HAAs have been the objective of numerous studies. It has been reported that the formation of HAAs was promoted by lipid oxidation, and the reactive carbonyl compounds are involved in the formation of HAAs[13–15]. However, the complete HAAs formation mechanism related to oxidation is still not fully established. Hence, it is necessary to study whether there is a correlation between lipid oxidation, protein oxidation and HAAs formation in smoked meat products, which will be helpful to develop some targeted strategies to control their formation in the smoked meat products.

Harbin red sausages are rich in fat and protein and are particularly favoured by consumers in northern China[16–17]. In our previous study, the presence of HAAs in Harbin red sausage was confirmed, with detection levels reaching hundreds of nanograms

per gram, depending on the smoking method and smoking material[18]. Therefore, in order to further study the factors affecting the formation of HAAs in smoked meat products from the perspective of smoking materials, the purpose of this study was to investigate the HAAs level in Harbin red sausages smoked using different species of wood chips (pear, oak, apple, beech). In addition, the relationships among moisture content, water activity (a_w), pH, lipid oxidation level and protein oxidation level with the HAA contents of sausages were established based on partial least-squares regression (PLSR). The information derived from this study may reveal clues to understanding how the origin of the wood affects HAA formation in smoked meat products and provide a useful theoretical basis for the industrial production of smoked meat products to control the formation of HAAs.

2 Materials and methods

2.1 Chemicals

For use in high-performance liquid chromatography (HPLC) analysis, acetonitrile, dichloromethane, *n*-hexane and acetic acid were obtained in HPLC-grade from Fisher Scientific (Loughborough, UK). The chemical standards of the HAAs studied, namely 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ), 2-amino-9*H*-pyrido[2,3-*b*] indole (AαC), 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoxaline (IQx), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*] indole (MeAαC), 2-amino-3,4-dimethylimidazo-[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethyl-3*H*-imidazo[4,5,*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo-[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-3,4,7,8-tetramethyl-3*H*-imidazo[4,5-*f*] quinoxaline (4,7,8-TriMeIQx), 2-amino-3,7,8-trimethyl-3*H*-imidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), 2-amino-5-phenylpyridine (Phe-P-1), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 1-methyl-9*H*-pyrido[3,4-*b*]indole (Harman) and 9*H*-pyrido-[3,4-*b*]indole (Norharman) were purchased from Toronto Research Chemicals (Toronto, Canada).

2.2 Sausage preparation

Three independent batches of Harbin red sausages were prepared (replicates) on the different days. The sausages were prepared in accordance with the formula described by Lv et al.[19]. A basic formula was prepared for each group of sausages: 1 500 g lean pork, 380 g pork back fat, 120 g starch, 56.4 g salt, 0.15 g sodium nitrite, 0.75 g sodium erythorbate, 4.5 g alkaline phosphate, 4.0 g monosodium glutamate, 10.0 g garlic, 4.0 g ground pepper, and 500 g ice water. Pork back fat and lean pork, minced through a 4-mm plate previously, were cured with a curing agent (salt, sodium nitrite, sodium erythorbate, sodium nitrite and alkaline phosphate) and salted (salt) at 4 ℃ for 20 h, separately. The ground mixture was then mixed thoroughly in a mixer with the other ingredients, according to the formula. After that, the mixture was stuffed in a natural casing (porcine intestine, 38 mm in diameter), and sausages were cooked at 85 ℃ for 30 min and roasted at 70 ℃ for 40 min in an automatic smokehouse. The cooked sausages were immediately divided into five equal portions (treatments): one treatment was unsmoked (control), and the other four treatments were smoked using pear, apple, oak and beech wood chips at 65 ℃ for 2 h, respectively. The apple, pear, oak, and beech wood chips were selected because they are commonly used wood species for smoked meat products, which can impart pleasant flavour to the smoked meat products[20]. The contents of cellulose, hemicellulose and lignin of pear, apple, oak and beech wood chips were showed in our previous study[20], which were determined according to the AOAC method[21]. Each treatment used approximately 1.5 kg of wood chips. After sausage preparation, the sausages were analysed within one day. All analyses were performed in triplicate.

2.3 Moisture content, a_w and pH

Moisture content was measured by drying 5 g samples until the weight variation between two successive measurements was < 0.1% according to the Association of Official Analytical Chemists (AOAC) method[21]. Measurements of a_w were performed at 25 ℃ using a water activity meter (Aqualab 4TE, Decagon Devices, Inc., Pullman, WA, USA). For pH measurement, a slurry was prepared by mixing 10 g of each sample and 90 mL of distilled water, and the pH was recorded using a standard pH meter (Mettler Toledo Instruments Co., Ltd., Shanghai, China), as described by Chen et al.[22].

2.4 Determination of thiobarbituric acid-reactive substances (TBARs) and carbonyl content

Lipid oxidation levels were evaluated by the TBARs method described by Chen et al.[23]. Briefly, 2.00 g minced samples were homogenized with 3.0 mL of 1% thiobarbituric acid solution and 17 mL 2.5% trichloroacetic acid-hydrochloric acid (TCA-HCl) solution. Then the mixture was heated in a boiling water bath for 30 min. After being cooled to room temperature, 4 mL of the suspension was mixed with 4 mL of chloroform, followed by centrifugation at 3 000 × *g* for 10 min. Finally, the upper phase was measured at a wavelength of 532 nm. Results were expressed as milligrams of malondialdehyde (MDA) per kilogram of sausage.

Protein carbonyl contents (nmol carbonyl/mg protein) were quantified by the 2,4-dinitrophenylhydrazine (DNPH) method reported by Estévez et al.[24] with minor modifications. Minced samples (3.00 g) were homogenized with 15 mL phosphate buffer (10 mmol/L, pH 7.4) for 30 s, then filtered with qualitative filter papers (Whatman No. 2). Two equal aliquots of 0.5 mL filtrate and 0.5 mL 10% TCA were mixed and centrifuged at 3 500 × *g* for 6 min, followed by washing twice with 0.5 mL hydrochloric acid/acetone (3:100, *V*/*V*). One pellet was mixed with 1 mL 2 mol/L HCl in order to measure protein concentration, while the other pellet was mixed with 1 mL of 0.2% (*m*/*V*) DNPH in 2 mol/L HCl in order to measure carbonyl concentration. Both tubes were incubated for 1 h at room temperature (vortex oscillation every 15 min). Subsequently, 1 mL 20% TCA added into tubes and centrifuged at 10 750 × *g* for 5 min, the pellets were washed three times with 1 mL ethanol-ethyl acetate (1:1, *V*/*V*) to remove excess of DNPH. The pellets were mixed with 3 mL 6 mol/L guanidine HCl (pH 6.5) at 37 ℃ for 15 min, and then the sample was centrifuged at 10 750 × *g* for 3 min to remove the insoluble substance. Finally, the upper phase was measured at a wavelength of 370 nm for protein hydrazones. The carbonyl content was expressed as nmol/mg protein and calculated by an absorption coefficient of 22 000 L/(mol·cm).

2.5 HAAs quantitative analysis

HAAs were extracted and purified by adopting the methods reported by Yin et al.[18]. A UPLC system equipped with a triple quadrupole mass spectrometer (LCMS-8 050, Shimadzu Co.,

Kyoto, Japan) was used to identify and quantify the HAA contents. The Oasis MCX cartridge (60 mg/3 mL; Waters, Milford, MA, USA) was used for solid-phase extraction and a Shim-pack XR-ODS III reverse-phase analytical column (2.0 mm × 150 mm, 2.2 μm) was used for the separation process. The mobile phase consisted of 10 mmol/L ammonium acetate and ethyl acetonitrile. A known amount of solution of 4,7,8-TriMeIQx (used as an internal standard) was added to samples before they were injected into the LCMS. Each standard curve was acquired by plotting concentration ratio (standard vs. internal standard) against its area ratio.

The analysis of HAAs was supported by Shimadzu (China) Co., Ltd., in the Shenyang Analytical Centre. In this study, the regression coefficients (R^2) of the standard curves for the twelve HAAs were all higher than 0.99. The LOD and LOQ of the HAAs ranged from 0.010 9 to 0.107 5 ng/g and 0.033 1 to 0.325 7 ng/g, respectively. The recoveries of the HAAs varied between 60.02% and 91.70%, in agreement with previous reports from this laboratory[18].

2.6 Statistical analysis

Statistical analysis was carried out using the R software (version 3.4.3, Auckland, New Zealand). Differences between means were determined by the Tukey test at $P < 0.05$. Results were presented as mean ± standard error (SE). Variations in the HAA contents and physicochemical properties were analysed by a mixed procedure treating wood species as a fixed effect and sample and preparation day as random effects. The associations between HAA contents, lipid oxidation levels, protein oxidation levels, moisture contents, a_w and pH were examined by PLSR.

3 Results and discussion

3.1 Moisture content, a_w and pH of sausages

The moisture content, a_w and pH of sausages smoked using different wood species are presented in Table 1. Significantly lower moisture contents were recorded in the smoked samples than the unsmoked sample ($P < 0.05$) due to the moisture loss during the smoking process[25]. In addition, the denaturation of proteins during the smoking process reduced their water-holding capacity, and accelerates the evaporation of water molecules[26]. The pH ranged from 6.08–6.16. Similar results were also reported by Hitzel et al.[27], who evaluated the pH value in sausages smoked with different types of woodchips (beech, oak, spruce, poplar, alder, hickory, and fir). Their results showed that pH values ranged from 6.05 to 6.26. The sausages smoked using wood chips from pear, beech, and oak had significantly lower pH values compared to the unsmoked sample ($P < 0.05$), in agreement with other studies that the smoking process will also influence the pH of the final product[28–29]. In general, large quantities of organic acids have been identified in both the vapor and the particulate phases of wood smoke, which are known to decrease the pH on the surface of the samples[30]. There were no significant differences in the moisture content (59.77%–60.88%), a_w (0.958–0.963) and pH (6.08–6.13) among the smoked samples ($P > 0.05$).

3.2 Lipid and protein oxidation levels

TBARs values mainly reflect the degree of lipid oxidation. The TBARs in sausages smoked using different species of wood are illustrated in Figure 1A. There was less TBARs detected in the unsmoked sample (approximately 0.25 mg MDA/kg) than in the smoked samples ($P < 0.05$), which exhibited TBARs values of 0.49, 0.43, 0.39 and 0.35 mg MDA/kg sausage using wood chips from pear, apple, oak and beech, respectively. The results agreed with Huang et al.[31] that lipid oxidation increases during the smoking process. All TBARs values were lower than the generally acceptable sensory threshold of 0.5–1.0 mg MDA/kg[32]. Compared to the previous studies, the current TBARs values of the samples smoked using different species of woods were lower than the result obtained by Malarut et al.[11] at 0.81 mg MDA/kg, but higher than the result obtained by Kim et al.[33] at 0.12 mg MDA/kg. The TBARs value of the sausages smoked with pear wood chips was significantly higher than those sausages smoked by beech and oak wood chips ($P < 0.05$). Similar results were also reported by Malarut et al.[11], who determined the TBARs values of sausages smoked with different types of woodchips, and found beech-smoked sausage had the lowest oxidation value. These results indicated that the lipid oxidation in smoked sausages was affected by the woodchip type. Pöhlmann et al.[34] reported that phenolic compounds were formed by pyrolysis of woodchips, and those compounds may have some antioxidant activity.

The formation of carbonyl compounds is widely recognised as a marker of protein oxidation[13]. In general, the content of total protein carbonyls in meat and meat products ranges from 0.5–1.0 nmol/mg protein in raw meat to approximately 20 nmol/mg protein in processed meat[8,35–36]. The carbonyl contents found in sausages smoked using different wood species are illustrated in Figure 1B. Similar to the TBARs values, the carbonyl content was significantly lower in the unsmoked sample than smoked samples ($P < 0.05$). The carbonyl content of the samples smoked using different wood species was in the range of 3.43–3.94 nmol/mg protein, with significantly greater amounts observed when apple and pear wood chips were used rather than beech and oak wood chips, respectively ($P < 0.05$). Consistent with this result, Soladoye et al.[8] reported that most carbonyl compounds in bacon were produced during the smoking process, with the lowest total carbonyl content of 80.01 nmol/mg protein found in the control bacon. The increased carbonyl content after smoking may be due to the fact that some basic amino acids (lysine, histidine and arginine) are easily attacked by free radicals generated during the smoking process, facilitating their conversion into carbonyl derivatives[8]. Therefore, the smoking process could largely contribute to the high level of protein oxidation in meat products.

Table 1 Moisture content, a_w and pH of Harbin red sausages smoked using different wood species.

Index	Control	Pear	Oak	Apple	Beech
Moisture content (%)	64.52 ± 0.47^a	60.88 ± 0.40^b	60.46 ± 0.31^b	60.15 ± 0.43^b	59.77 ± 0.02^b
a_w	0.968 ± 0.001^a	0.959 ± 0.001^b	0.960 ± 0.001^b	0.963 ± 0.001^{ab}	0.958 ± 0.002^b
pH	6.16 ± 0.01^a	6.10 ± 0.01^b	6.08 ± 0.01^b	6.13 ± 0.02^{ab}	6.09 ± 0.01^b

Note: Different lowercase letters (a–c) in the same row indicate significant differences among different samples ($P < 0.05$).

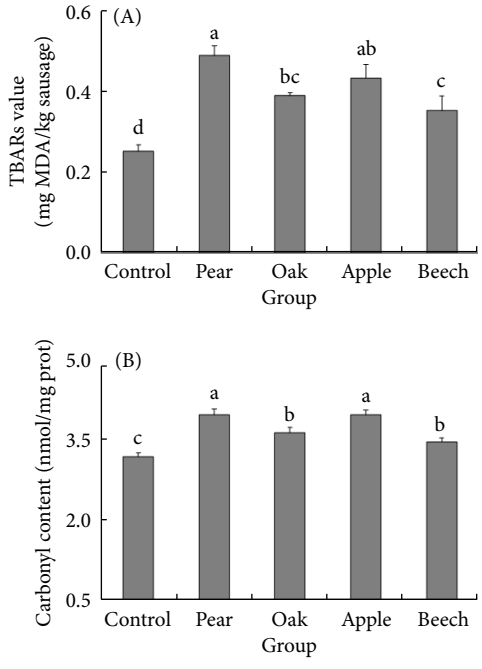

Figure 1 (A) TBARs value and (B) carbonyl content of Harbin red sausages smoked using different species of wood. Means with different lowercase letters differ significantly ($P < 0.05$).

3.3 HAA content

The levels of polar HAAs and non-polar HAAs in sausages are illustrated in Figure 2. In this study, four polar HAAs (IQ, IQx, MeIQ and 7,8-DiMeIQx) and three non-polar HAAs (MeAαC, Harman and Norharman) were found. As shown in Figure 2A, the total HAAs level was significantly higher in the smoked samples than in the unsmoked sample ($P < 0.05$), which could be mainly related to the involvement of the smoke constituents in the generation of HAAs in the samples[1,10,37–38]. According to Gibis[1], the carbonyl compounds in smoke may provide intermediates of HAAs that promote the HAAs formation. In addition, the higher HAA contents could be due to the lower moisture content. Less water would have been expected to be available in the lower moisture condition, effectively creating a concentrated reaction system in which the formation of HAAs is accelerated[39–40]. The HAAs content was highest in the sample smoked using the pear wood chips (55.050 ng/kg), followed by the oak (40.122 ng/kg), beech (28.452 ng/kg) and apple wood chips (27.147 ng/kg), which showed that the woodchip types had a noticeable influence on the HAA contents in smoked sausages. Yang et al.[10] found that smoky ingredients may be involved in the formation of HAAs during the smoking process. However, there were no significant differences for total HAAs content between the samples smoked using beech and apple wood chips ($P > 0.05$). The total HAAs contents in Harbin red sausages in this study were much lower than those in smoked poultry products reported by Zhang et al.[41] and much higher than those in smoked lamb reported by Hou et al.[42]. These differences may due to the different smoking methods, duration, temperatures, and woodchip types[18].

All smoked samples had higher contents of non-polar HAAs (25.051–52.889 ng/kg, sum of MeAαC, Harman and Norharman) than polar HAAs (2.046–2.161 ng/kg, sum of IQ, MeIQ, IQx and 7,8-DiMeIQx). Norharman was found at the highest concentration among the HAAs in this study, followed by Harman. Both β-carbolines are widely found in cooked bacon and sausage[10,43]. It indicated that the primary accumulation in the smoking processing was of non-polar HAAs. In general, the non-polar HAAs are formed by the pyrolysis of amino acids at temperatures over 300 ℃, which are regarded as products of protein pyrolysis, although aldehydes have been identified as intermediate compounds of β-carbolines[44]. However, their detection in meat products smoked at relatively lower temperatures has been demonstrated[9–10]. According to Rönner et al.[45], the tryptophan Amadori rearrangement product was readily formed from tryptophan in the presence of glucose under heat-processed conditions, then it transformed to tetrahydro-β-carbolines in the presence of reactive carbonyl compounds and finally formed Norharman and Harman after oxidation. Thus, the presence of carbonyl compounds in wood smoke could facilitate the formation of non-polar HAAs[10]. As shown in Figure 2B, the content of Norharman in the smoked samples could be ranked by the wood species used as pear (44.784 ng/g) > oak (32.114 ng/g) > beech (20.242 ng/g) > apple (18.980 ng/g). This trend was slightly different from that of the Harman content: pear (6.934 ng/g) > apple (4.899 ng/g) > oak (4.763 ng/g) > beech (4.688 ng/g), but pear wood chips ranked first for both Norharman and Harman, which could, in part, be related to the relatively high carbonyl content (Figure 1B). It could also be related to the composition of the wood smoke produced by different wood species[25]. As our previous study showed, the pear wood chips contained a high content of cellulose, whose thermal decomposition could generate large contents of carbonyl compounds[11,20]. Overall, the woodchip types had a significant influence on the content of non-polar HAAs in sausages, which in turn influence the total HAA contents. From Figure 2C, the total content of polar HAAs was significantly lower in the unsmoked

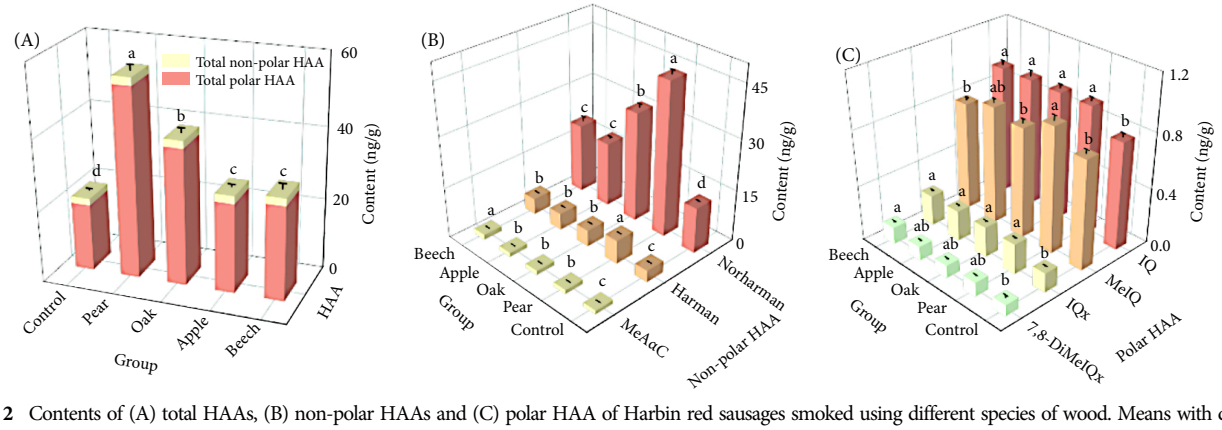

Figure 2 Contents of (A) total HAAs, (B) non-polar HAAs and (C) polar HAA of Harbin red sausages smoked using different species of wood. Means with different lowercase letters differ significantly ($P < 0.05$).

sample than the smoked samples ($P < 0.05$). The concentration range of MeIQ in the smoked samples was 0.782–0.914 ng/g, with higher contents ($P < 0.05$) found using wood chips from pear (0.914 g/g) than oak (0.804 ng/g) and beech (0.782 ng/g). IQ, IQx and 7,8-DiMeIQx were found in the ranges of 0.925–0.931, 0.219–0.220 and 0.091–0.120 ng/g, respectively, in the smoked samples, with no significant differences observed ($P > 0.05$).

3.4 Correlation analysis

The main aim of this study was to investigate the influence of different species of wood chips on the contents of HAAs. Although the precursors of HAAs are glucose, amino acids, proteins and creatine, some other factors, such as moisture, a_w, pH, lipid oxidation and protein oxidation, will also affect the formation of HAAs. The correlation between the X variables (physicochemical properties: moisture content, a_w, pH, TBARs value, carbonyl content) and Y variables (HAA content) of Harbin red sausages is shown in Figure 3. The X variables ($R^2_X = 0.934$) explained the variation in the Y variables ($R^2_Y = 0.571$) according to the first two factors ($P < 0.05$, $Q^2 = 0.403$). From Figure 3, moisture content, a_w and pH negatively correlated with all HAAs in the present research, in line with Zhang et al.[46]. According to Skog[47], water has a considerable influence on the formation of HAAs. Water is constantly evaporating during the cooking process. Water can act as a reaction and transfer medium, transporting precursors to the surface of the product where they are exposed to higher temperatures, and promoting the formation of HAAs[48].

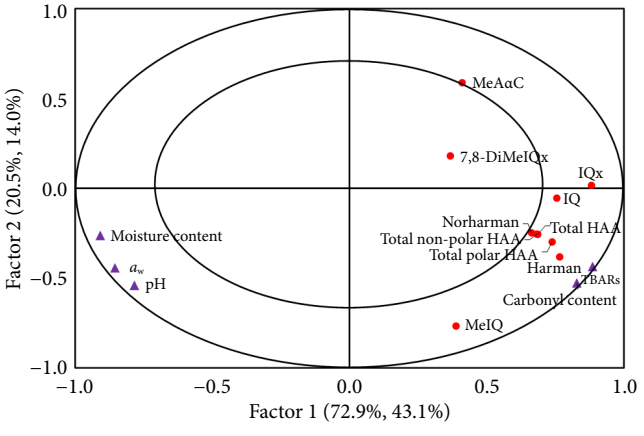

Figure 3 Correlation between physicochemical properties (moisture content, a_w, pH, TBARs value, carbonyl content) and heterocyclic aromatic amine content of Harbin red sausages.

In this study, positive correlations existed between the total HAAs and TBARs value and between the total HAAs and protein carbonyl content. Moreover, except for 7,8-DiMeIQx and MeAαC, the TBARs value and protein carbonyls, respectively, had a significant positive correlation with each HAA ($P < 0.05$). The Correlation results indicated that both lipid oxidation and protein oxidation contributed to the formation of HCAs during smoking process. Randel et al.[49] had proved that the formation of HAAs was promoted by lipid oxidation, and Zhang et al.[41] showed that reactive carbonyl compounds are involved in the formation of HAAs. Meanwhile, the results were in line with the report of Lu et al.[13], who found that lipid oxidation and protein oxidation were associated with the development of HAAs through interactions of radicals generated from lipid oxidation and the Maillard reaction. Aldehydes and ketones generated from peroxyl radicals in lipid oxidation could participate in the Maillard reaction and promote the formation of pyrazine and pyridine via reacting with amino acids[14]. Pyrazine and pyridine can also participate in the formation of polar HAAs[15]. At the same time, peroxyl radicals generated from lipid oxidation could also trigger protein oxidation[8]. The Maillard reaction, lipid oxidation and protein oxidation are interrelated, which could explain the significant correlation found in the current study.

4 Conclusion

This study determined the physicochemical properties and HAA levels in Harbin red sausages that were unsmoked and smoked using pear, apple, oak and beech wood chips. The total content of HAAs was significantly higher in the smoked samples than in the unsmoked sample. The sample smoked using pear wood chips contained the highest contents of individual and total HAAs, followed by the samples smoked using oak, beech and apple wood chips. Samples smoked using apple and beech wood chips, respectively, showed comparable HAA levels. In summary, the HAA formation of smoked meat products is significantly affected by the smoking process and wood type. The information derived from this study provides valuable insights into how the origin of the wood affects HAA formation in smoked meat products, and provides a useful theoretical basis for the industrial production of smoked meat products to control the formation of HAAs. Sausage processing conditions and the type of wood chips used for smoking should be carefully controlled to minimise the cancer risk of smoked meat products. Moreover, the results of the study can be used along with dietary assessments to estimate HAA exposure due to consumption of smoked meat products. In this study, the lipid and protein oxidation levels were highly correlated with the total HAA content in our study. However, the complete HAAs formation mechanism related to oxidation is still not fully established. Therefore, further studies should be devoted to elucidating the critical processes and underlying mechanisms involved in the relations reported regarding HAAs formation and protein and lipid oxidation in thermally processed meat products. And further research is needed to gain a better understanding of how dietary components interact with HAAs. Certain compounds found in foods, such as antioxidants and phenolic compounds, may help limit the formation of HAAs and decrease their harmful effects. Exploring the synergistic or antagonistic effects of these dietary components on HAAs formation will provide a foundation for future research aimed at implementing various control and inhibition strategies.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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