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Formation and network relationship of four hazards in fried foods

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ABSTRACT: Acrylamide (AA), 5-hydroxymethylfurfural (5-HMF), heterocyclic amines (HCAs), and polycyclic aromatic hydrocarbons (PAHs) are prevalent in fried foods and pose significant health risks. Our study explored the formation of these hazards in fried potatoes, tofu, and chicken wings across a range of frying temperatures. We discovered that elevated temperatures and extended oil heating durations substantially increased the formation of these hazards. Notably, AA and 5-HMF were most concentrated in potatoes, which are rich in reducing sugars, while HCAs and PAHs were particularly high in chicken wings, attributing to their high fat and protein content. Intriguingly, significant correlations were identified among the concentrations of these hazards. Further analysis unveiled a network of interconnected formation mechanisms, highlighting shared intermediates and pathways such as the Maillard reaction, lipid and protein oxidation, and sugar pyrolysis. These findings deepen our comprehension of how these hazards interrelate and form, offering valuable insights for developing strategies to reduce their concurrent formation during the frying process.

Keywords: Acrylamide; 5-Hydroxymethylfurfural; Heterocyclic amines; Polycyclic aromatic hydrocarbons; Frying; Network relationship

1. Introduction

Deep frying, an ancient culinary technique, utilizes oil to catalyze desirable physical and chemical transformations in food, conferring characteristics such as a distinctive flavor and a crispy texture that have made it a global favorite (Hosseini et al., 2016). Soybean oil is widely used as a frying oil in the food industry due to its cost-effectiveness and ability to meet nutritional requirements. This widespread adoption in food preparation has further enhanced its popularity among consumers (Napolitano, et al., 2018). Despite deep frying benefits, this method can also result in the production of food hazards like acrylamide (AA), 5-hydroxymethylfurfural (5-HMF), heterocyclic amines (HCAs), and polycyclic aromatic hydrocarbons (PAHs), which are associated with serious health issues, including neurotoxicity, reproductive harm, genetic mutations, and carcinogenicity (Bulanda & Janoszka, 2022). Consequently, examining the formation of these hazards during the frying process is imperative for safeguarding food safety and public health.

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The International Agency for Research on Cancer (IARC) has catalogued four primary food hazards, detailing their molecular structures, weights, and carcinogenic classifications in Table S1. Notably, Acrylamide (AA) is classified as a Group 2A carcinogen, typically arising in foods abundant in carbohydrates and deficient in protein through the Maillard reaction (Başaran & Turk, 2021). Additionally, acrolein, originating from lipid-rich foods, is recognized for its role in AA formation (Stadler & Scholz, 2004). Conversely, 5-HMF ranks as a Group 3 carcinogen by IARC, reflecting its indeterminate carcinogenic potential. It forms via the Maillard reaction or sugar caramelization under acidic conditions (Locas & Yaylayan, 2008). Studies by Gökmen et al. (2012) have shown that 5-HMF quickly reacts with asparagine to foster AA formation, positioning 5-HMF and related carbonyls as significant contributors to AA formation in thermally processed foods. Heterocyclic Amines (HCAs), often found in protein-rich foods during heat treatment due to interactions involving creatine and creatinine, consist mostly of Group 2B and Group 3 carcinogens (Zhou et al., 2022). Polycyclic Aromatic Hydrocarbons (PAHs), notably benzo[a]pyrene (BaP)—a Group 1 carcinogen—predominantly originate from heat-treated foods and are highly hydrophobic, tending to accumulate in the food chain (Zhang et al., 2021). Given that current research predominantly focuses on individual hazards, there is a knowledge gap regarding their concurrent formation and interactions during thermal processing. Unraveling the interwoven formation pathways of AA, 5-HMF, HCAs, and PAHs through a network relationship is essential for grasping their complex correlations and interactions.

Globally, the most popular fried foods are potatoes, tofu, and chicken wings, each contributing unique nutritional profiles: potatoes offer plentiful calories from starch; tofu is prized for its healthful lipids and bioactive compounds (Ali et al., 2021); and chicken wings are a popular source of high-quality animal protein. Prior research has often isolated single hazards in specific foods, such as AA and 5-HMF in potatoes (Miao et al., 2014) or HCAs and PAHs in fried meats (He et al., 2022), with scant data on fried tofu. Given the distinct compositions of these foods, they present a promising model for studying hazard formation during frying. Hazard formation and accumulation are influenced by various factors, including frying temperature and time, underscoring the need for a comprehensive investigation of these factors across different frying conditions.

The aim of this study was to map the formation patterns of AA, 5-HMF, HCAs, and PAHs in potatoes, tofu, and chicken wings during deep frying at various temperatures. Additionally, we assessed how these hazards dynamically change in relation to each other across different foods and temperatures. By establishing a network based on food components, we elucidated the mechanisms behind their formation and interactions, offering a scientific foundation for mitigating multiple food hazards during the frying process.

2. Materials and methods

2.1 Chemicals and materials

AA (purity, 99.6%) and 5-HMF (purity, 99%) were obtained from ANPEL-TRACE Standard Technical Services Co., Ltd. (Shanghai, China). The standard HCA mixture consisted of

2-amino-3,4-dimethyl-3H-imidazo[4,5-f]quinoline (MeIQ), 2-aminodipyrido[1,2-a:3',2'-d]imidazole dihydrochloride (Glu-P-2), 2-amino-3-methyl-3H-imidazo[4,5-f]quinoxaline (IQx), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole hydrochloride hydrate (Glu-P-1), 2-amino-3,4,8-trimethyl-3H-imidazo[4,5-f]quinoxaline (4,8-DiMeIQx), 2-amino-3,7,8-trimethyl-3H-imidazo[4,5-f]quinoxaline (7,8-DiMeIQx), 2-amino-3,4,7,8-tetramethyl-3H-imidazo[4,5-f]quinoxaline (4,7,8-TriMeIQx), 9H-pyrido[3,4-b]indole (Norharman), 1-methyl-9H-pyrido[3,4-b]indole (Harman), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole acetate (Trp-P-1), and 2-amino-9H-pyrido[2,3-b]indole (AαC; 100 $\mu g \cdot mL^{-1}$ of each) in methanol, was purchased from Toronto Research Chemicals (Toronto, Canada). The standards of benz[*a*]anthracene (BaA, purity, 99%), chrysene (Chr, purity, 99%), and benzo[*b*]fluoranthene (BbF, purity, 98%) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). BaP (purity, 99%) was obtained from Sigma-Aldrich (Missouri, United States). Glyoxal was an analytical grade reagent and acquired from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Ethanol, ether, petroleum ether, n-hexane, concentrated ammonia water, concentrated hydrochloric acid, methylene blue, methylene chloride, acetone, toluene, potassium hexacyanoferrate (II) trihydrate, zinc sulfate heptahydrate, zinc acetate, anhydrous sodium sulfate, copper sulfate, potassium sulfate, sodium potassium tartrate, sodium hydroxide, o-phenylenediamine, boric acid, and petroleum ether (with a boiling range of 30 °C to 60 °C) were analytical grade reagents, while acetonitrile, methanol, formic acid, acetic acid, and ammonium acetate were chromatography grade, and all of them purchased from Sinopharm (Shanghai, China). The HC-C18 SPE cartridge (10 mL/2 g) and Si SPE cartridge (6 mL/1 g) were acquired from CNW Technologies GmbH (Darmstadt, Germany). The Agela Cleanert PCX SPE cartridge (3 mL/60 mg) was obtained from Agela Technologies (Tianjin, China).

2.2 Preparation of fried foods

Soybean oil, potatoes, tofu, and chicken wings were purchased from a local supermarket (Yangzhou, China). The potatoes were cleaned, peeled, and cut into cubes (5 cm × 1 cm × 1 cm). Tofu was cut into rectangles (4 cm × 4 cm × 1 cm) and chicken wings were scratched on the surface. The food to be fried was rinsed with deionized water and then dripped. Soybean oil (3 L) was heated in an electric fryer (EF-82, Guangzhou Yingliansite Catering Equipment Co., Ltd, China) to specified temperatures (150, 170, and 190 °C) respectively, and approximately 100 g of each food type was fried per batch. The frying time of potatoes, tofu, and chicken wings was set at 4, 4, and 7 min, respectively. Each type of food was fried separately, with oil changes between different conditions. A batch of each food was fried every 30 min at each temperature, while the oil remained at 180 °C during intervals, with a total usage time of 10 h. Fried food samples were collected in sealed bags every 2 h, and then stored at -20 °C after cooling to room temperature. Before the determination of hazards, samples were vacuum freeze-dried at -60 °C for 48 h and evenly crushed into powder.

2.3 Determination of basic components of fried foods

The moisture, ash, protein, fat, and reducing sugar contents of raw foods were determined according to the Chinese National Standards (GB 5009.3—2016, GB 5009.4—2016, GB 5009.4—2016, GB 5009.5—2016 and GB 5009.6—2016). Briefly, moisture content was determined by oven-drying at 105 °C until constant weight. The determination of ash was obtained by burning samples at (550 ± 25) °C in a high-temperature furnace. Protein content was determined by semi-automatic Kjeldahl equipment (KDN-04III, Shanghai Xianjian Instrument Co., Ltd, China). The conversion coefficient of nitrogen to protein in potatoes and chicken wings was 6.25, while that of tofu was 5.71. The fat content was determined by petroleum ether Soxhlet extraction. Reducing sugar content was calculated based on the volume of sample titrated with calibrated alkaline copper tartrate under heating conditions with methylene blue as the indicator.

2.4 Determination of AA

The determination of AA content was based on Wang et al. (2013) with a slight modification. The sample powder (2.0 g) was weighed with 20 mL ultra-pure water using ultrasonic extraction for 30 min, followed by centrifugation at 8000 r/min for 10 min. The supernatant was mixed with 10 mL n-hexane, shaken, and allowed to stratify. Carrez I and II reagents (prepared with potassium hexacyanoferrate (II) trihydrate and zinc sulfate heptahydrate, respectively) were added to the degreased extract, centrifuged to remove proteins, and then purified using an HC-C18 SPE cartridge. The analyte was eluted with acetonitrile/water (5:95, V/V), dried by nitrogen blowing at 60 °C, dissolved in 1 mL ultra-pure water, filtered, and analyzed by HPLC with a Diamonsil C18 column (5 μm, 250 mm × 4.6 mm, Beijing Dicoma Technology Co., LTD., Beijing, China) at 30 °C. The mobile phase was a mixture of 95% (V/V) ultra-pure water containing 0.1% (V/V) formic acid and 5% (V/V) acetonitrile at a flow rate of 0.6 mL/min. The ultraviolet (UV) detector was used and the absorption wavelength was set at 210 nm.

2.5 Determination of 5-HMF

5-HMF extraction and purification followed similar procedures to AA, with methanol as the eluent. The eluate was dried under nitrogen at 40 °C, dissolved in 1 mL methanol, and analyzed by HPLC-UV using methanol/0.1% formic acid water (10:90, V/V) as the mobile phase at a flow rate of 1 mL/min. The detection was performed at 284 nm (Zou et al., 2015).

2.6 Determination of HCAs

HCAs content was determined using the method of Zhang et al. (2020) with slight modifications. Sample powder (2.0 g) was extracted with 10 mL acetonitrile containing 1% ammonia, centrifuged at 4 °C at 8000 r/min for 5 min, and repeated twice. The supernatant was degreased with acetonitrile-saturated n-hexane and purified using an Agela Cleanert PCX SPE cartridge. The analyte was eluted with methanol/ammonia water (19:1, V/V), dried under nitrogen at 45 °C, dissolved in 1 mL methanol, and analyzed by HPLC. The binary mobile phase system consisted of (A) 0.05 mol/L acetate/amine buffer (pH 3.6) and (B) acetonitrile. The mobile phase gradient program was set to 90% A and 10% B from 0 to 10 min, 80% A and 20% B from 10 to 25 min, 50% A and 50% B from 25 to 35 min, and finally to recover the initial composition 90% A and 10% B at 35 min. The flow rate of the mobile phase was 1 mL/min, and the column temperature was 30 °C. The UV detector was set at 263 nm for MeIQ, IQx, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, and 4,7,8-TriMeIQx. The fluorescence detector was programmed according to excitation/emission wavelengths from 0 min (360 nm/450 nm) for Glu-P-2 and Glu-P-1 to 10 min (300 nm/440 nm) for Norharman and Harman, followed by excitation/emission wavelengths for Trp-P-1 and PhIP to 17 min (315 nm/390 nm) and excitation/emission wavelengths of AαC to 21 min (335 nm/410 nm).

2.7 Determination of PAHs

The details of the extraction and clean-up procedures followed those in a previous study (Shen et al., 2022). Sample powder (2.0 g) was ultrasound-extracted thrice with dichloromethane, dried under nitrogen at 35 °C, and re-extracted with acetonitrile/acetone (3:2, V/V). The extract was concentrated under nitrogen, dissolved in 1 mL n-hexane, and purified using a Si SPE cartridge. The analyte was eluted with n-hexane and n-hexane/toluene (1:1, V/V), dried under nitrogen, and diluted with methanol. PAHs analysis was conducted using HPLC with an Athena PAHs column (4.6 mm × 250 mm, 5 μm) and a mobile phase of acetonitrile/water. The fluorescence detection was performed using the following excitation and emission wavelength programs: 270 nm/385 nm from 0 to 10 min (BaA and Chr), 256 nm/446 nm from 10 to 17 min (BbF), 292 nm/410 nm from 17 to 25 min (BaP).

2.8 Determination of glyoxal

The glyoxal in samples was determined by using the method reported by Weigel et al. (2004) with slight modification. In summary, the crushed sample (2.0 g) was extracted with 10 mL ultra-pure water using ultrasonic extraction, centrifuged, mixed with 10% o-phenylenediamine methanol solution, incubated at room temperature for 12 h, filtered, and analyzed by HPLC with a UV detector. A gradient elution program with the mobile phase comprising solvents A and B at a flow rate of 1.0 mL/min was used. Solvent A was ultra-pure water containing 0.1% (V/V) acetic acid, and solvent B was pure methanol. The elution program was applied as follows: 72% A (28% B) for 15 min, decreased to 57% A (43%B) from 15 to 30 min, and continuously decreased to 25%A (75%B) from 30 to 35 min, then kept for 10 min. The absorption wavelength of the UV detector was set at 314 nm.

2.9 Statistical analysis

All experiments were repeated three times. Excel 2019 (Microsoft, Redmond, WA, USA) and Origin 2021 (OriginLab, Co., Ltd, Northampton, MA, USA) were used for data statistics and graphing, and the data were expressed by mean ± standard deviation. SPSS 26.0 (International Business Machines Corporation, Armonk, NY, USA) was used for significance analysis ($P < 0.05$), and the correlation among hazards was evaluated by Pearson's correlation test.

3. Results and discussion

The basic components of potatoes, tofu, and chicken wings were analyzed and shown in Table S2. The moisture content of potatoes, tofu, and chicken wings was predominant among the basic components, especially tofu, which contributed to the formation of a crispy texture after the moisture evaporated during frying. Among these foods, chicken wings had the highest content of protein (15.68%) and fat (21.17%), while potatoes had the highest content of reducing sugar (0.19%). The differences in basic components of these foods may affect the concentrations of AA, 5-HMF, HCAs, and PAHs during frying.

3.1 Changes in AA concentration during frying

The changes in AA concentration of chicken wings at different frying temperatures (150, 170, and 190 °C) are shown in Fig. 1A. Higher frying temperatures and longer heating times promoted the formation of AA in chicken wings, with temperature having a more pronounced effect than time. This is consistent with the findings of Mariotti-Celis et al. (2017), who reported that frying temperature has a critical impact on the production of AA in French fries. As the increase of frying temperatures, the formation of lipid degradation products containing three carbons in chicken wings gradually increased, resulting in the continuous formation of acrolein, which would further be oxidized to acrylic acid and subsequently react with ammonia in chicken wings to form AA (Kuek et al., 2020).

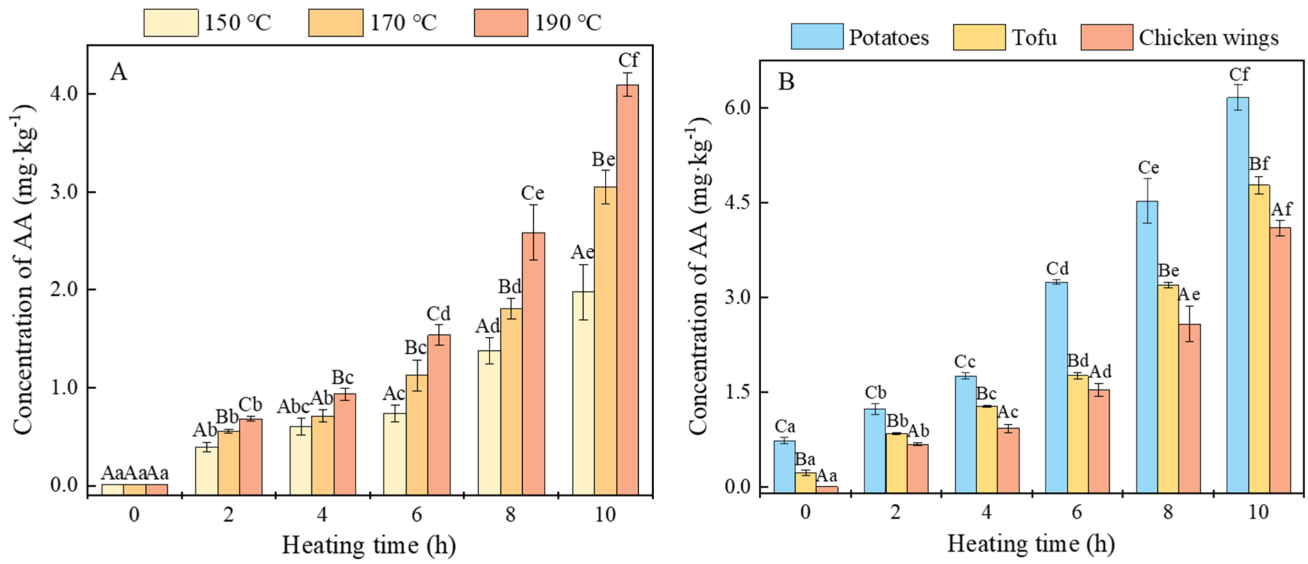

Fig. 1 Changes in AA concentration in chicken wings at different temperatures (A) and in different foods at 190 °C (B) during frying. Different capital letters (A-C) indicate significant differences among the different groups within the same heating time ($P < 0.05$). Different lowercase letters (a-f) indicate significant differences among the same groups within different heating time ($P < 0.05$).

Furthermore, the formation of AA in different fried foods was investigated at 190 °C (Fig. 1B). AA was detected in raw potatoes, tofu, and chicken wings, with the concentration of AA in potatoes (0.73 $mg \cdot kg^{-1}$) being much higher than in tofu (0.23 $mg \cdot kg^{-1}$) and chicken wings (0.01 $mg \cdot kg^{-1}$). This may be due to the degradation of polyacrylamide in the soil to AA (Paz-Alberto et al., 2011), resulting in residual AA in potatoes grown in soil treated with polyacrylamide. The concentration of AA in fried tofu and chicken wings ranged from 10 to 4775 $\mu g \cdot kg^{-1}$, and in chicken wings was lower. In fried potatoes, the measured concentration of AA was between 734 and 6152 $\mu g \cdot kg^{-1}$, significantly higher than in tofu and chicken wings, exceeding the threshold set by IARC (300—850 μg·kg^{-1}) (Tomatis et al., 1977). The high concentration of AA in fried potatoes was associated with their high content of reducing sugar (Table S2), as reducing sugars can react with asparagine (a free amino acid in potatoes) to form AA (Miao et al., 2014). Therefore, selecting potatoes with low reducing sugar content is recommended for frying. Furthermore, the concentration of AA in these three fried foods increased with the number of frying cycles. It has been reported that the levels of primary and secondary lipid oxidation products increased in oil after consecutive frying, which could react with asparagine to form AA (Hidalgo et al., 2010). Therefore, the changes in oil caused by continuous frying may be responsible for the increase in AA concentration.

Based on these results, the changes in AA concentration in foods were associated with frying temperature, the heating time of oil, and food components. Therefore, it is an effective way to reduce the formation of AA by controlling frying conditions and selecting appropriate foods for frying.

3.2 Changes in 5-HMF concentration in fried potatoes

In contrast to AA, 5-HMF was undetected in both raw or fried tofu and chicken wings, likely related to the low content of reducing sugar in these foods. 5-HMF easily forms in heated sugar-rich foods due to the high temperature promoting sugar decomposition and the Maillard reaction (Martins et al., 2022).

It can be observed from Fig. 2 that the concentration of 5-HMF increased significantly after frying compared to the low concentration in raw potatoes (0.04 mg·kg^{-1}). Moreover, the concentration of 5-HMF increased gradually with heating time, indicating that increasing the number of oil cycles significantly increased the concentration of 5-HMF accumulated in fried potatoes. This suggested that the formation of 5-HMF may be related to the oxidation of the oil. Frying potatoes in batches promotes the accumulation of lipophilic intermediate products, such as 3-deoxyglucosone (3-DG) and 3,4-dideoxyglucosone (3,4-DGE), in the oil, leading to the formation of 5-HMF in large quantities (Huang et al., 2022). The formed 5-HMF was more likely to migrate and accumulate in fried potatoes due to its hydrophilicity. Notably, it has been found that the concentration of 5-HMF in oil used for frying dough increased with the frying temperature and the number of frying (Göncüoğlu & Gökmen, 2013). This may be owing to the partial hydrophobicity of 5-HMF and the substance exchange between frying oil and food. 5-HMF may migrate to the oil when formed in fried food, leading it detectable in the oil. Our results remind people that the concentration of 5-HMF in fried foods with high reducing sugar content should not be ignored.

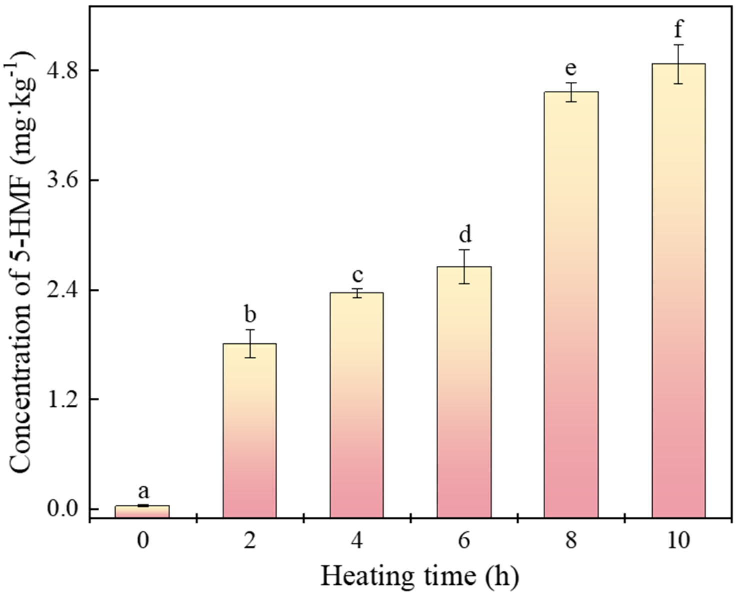

Fig. 2 Change in 5-HMF concentration in potatoes at 190 °C during frying. Different lowercase letters (a-f) indicate significant differences among the same groups within different heating time ($P < 0.05$).

In addition, the concentration of 5-HMF in fried potatoes was higher than that of AA only at the initial stage of oil heating, which may be because 5-HMF was formed earlier than AA in the Maillard reaction, and 5-HMF was one of the precursors for AA formation. As the reaction proceeded, 5-HMF would further react with asparagine to form AA (Gökmen et al., 2012).

3.3 Changes in HCAs concentration during frying

The dynamic changes in various HCAs concentration in fried chicken wings are shown in Fig. 3 (A, B, and C). Four polar HCAs, including MeIQ, MeIQx, 4,8-DiMeIQx, and 7,8-DiMeIQx, as well as three non-polar HCAs, Norharman, Harman, and PhIP, were detected in fried chicken wings, where the concentration of non-polar HCAs was higher than that of polar HCAs. The concentration of the seven HCAs increased significantly with an increase in frying temperature and oil heating time. At a low frying temperature (150 °C), MeIQ was not detected in chicken wings and MeIQx was not detected during 0–6 h of oil heating. Similarly, MeIQ was not detected in fried chicken wings at 170 °C from 0 to 4 h. These results showed that the formation of HCAs was affected by the frying temperature and heating time of the oil. Large quantities of oxides and free radicals were formed in the oil with repeated use, which promoted the formation of HCAs in fried chicken wings (Bulanda & Janoszka, 2022). In addition, the HCAs concentration in fried chicken wings increased significantly with the number of oil circulation, which was consistent with the research results of Li et al. (2023) on fried chickens.

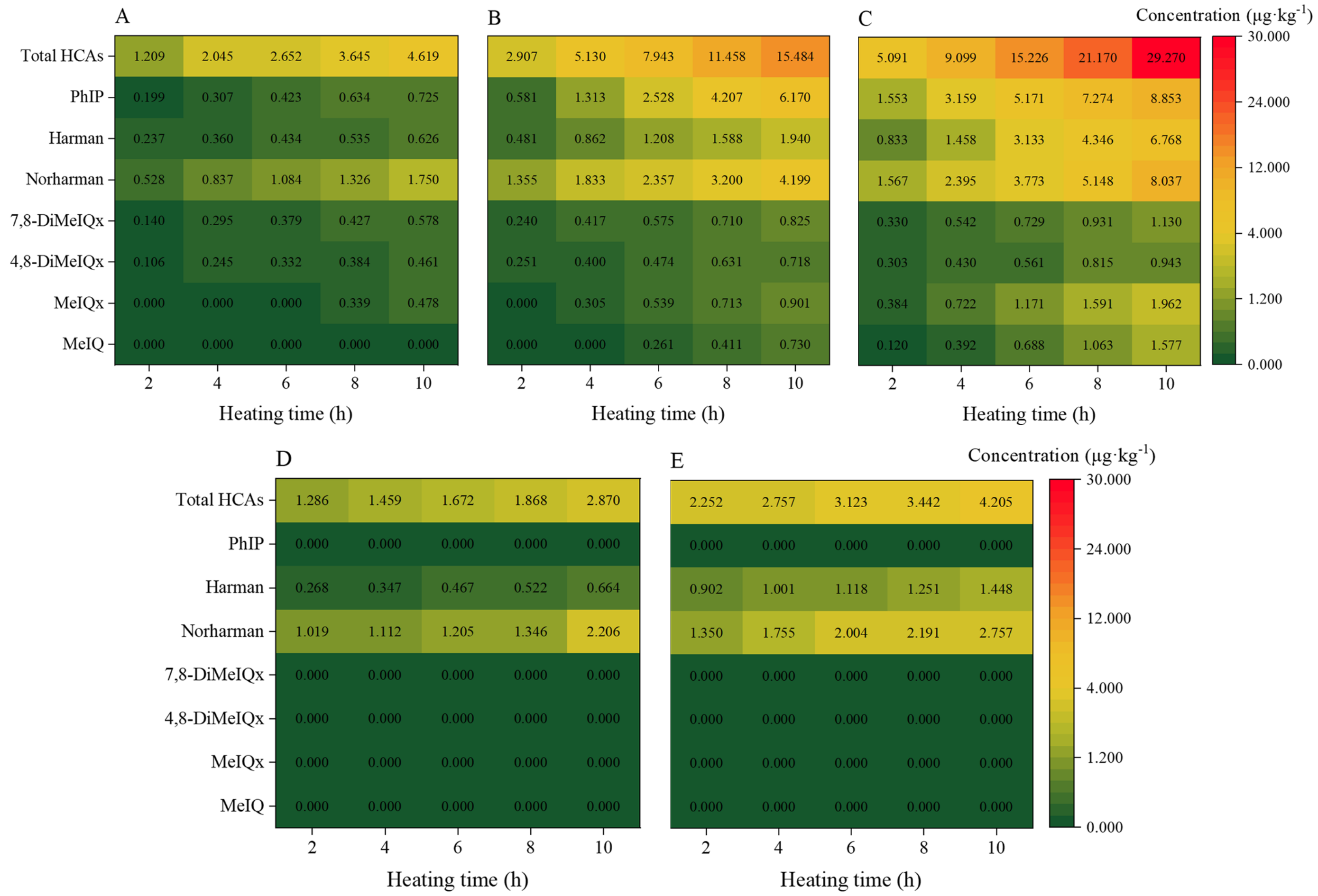

Fig. 3 Changes in HCAs concentration in chicken wings at different temperatures (A-C) and in different foods at 190 °C (D-E) during frying. Chicken wings fried at 150 °C (A), 170 °C (B), and 190 °C (C). Potatoes (D) and tofu (E) fried at 190 °C.

Fig. 3 (D, E, and C) shows the variation in the concentration of HCAs in fried potatoes, tofu, and chicken wings with the heating time of oil. Only two non-polar HCAs (Harman and Norharman) were detected in potatoes and tofu. Polar HCAs and PhIP were not detected in potatoes and tofu because of the lack of animal-derived creatine, which can react with free amino acids and sugar to form polar HCAs or PhIP (Elbir & Oz, 2021). The concentration of Harman and Norharman gradually increased with oil heating time, and it in chicken wings was higher than that in tofu, and it in potatoes was the lowest. The formation of Harman and Norharman may be related to the content of fat in food. A large number of aldehydes, ketones, acids, and reactive carbonyl compounds produced by the oxidative degradation of fat during frying promote the formation of Harman and Norharman (Herraiz, 2000). Compared with potatoes and tofu, more varieties and higher concentration of HCAs were formed in fried chicken wings, probably due to the higher levels of amino acids in chicken wings, in addition to the higher levels of creatinine and fat. Many amino acids may be degraded to produce HCAs during heating, such as serine, threonine, phenylalanine, and leucine to MeIQx and PhIP, tryptophan to Harman and Norharman, and so on (Dong et al., 2020).

3.4 Dynamic changes in PAHs concentration during frying

PAH4 (BaA, Chr, BbF, and BaP) has been recommended to use as the detection index of PAHs in food by the European Food Safety Authority (EFSA) because of the high frequency and strong carcinogenicity (Shen et al., 2022). PAH4 was detected in both raw and fried samples. Fig. 4 (A) shows the concentration of

PAH4 in chicken wings at different temperatures and oil heating times. The results showed that the frying temperature and oil heating time have a significant effect on the concentration of PAH4 in chicken wings. It was observed that the concentrations of BaA, Chr, and BbF in the fried chicken wings at 150 °C and 170 °C wavelike increased within 10 h, while the concentration of BaP increased continuously. The concentration of PAH4 in chicken wings at 190 °C continued to increase until the end of frying, and the total concentration of PAH4 increased by 0.76 μg·kg^{-1} compared with the initial concentration. The decrease in PAHs concentration in chicken wings during continuous heating of the oil may be related to the migration of PAHs into the oil fume during frying, as well as the migration of PAHs from the less lipophilic substrate (chicken wings) to the more lipophilic substrate (oil) during frying (Iwegbue et al., 2020). On the contrary, the increase in PAHs concentration during frying may be linked to the increase in PAHs concentration in oil and the material exchange between oil and fried food (Li et al., 2016).

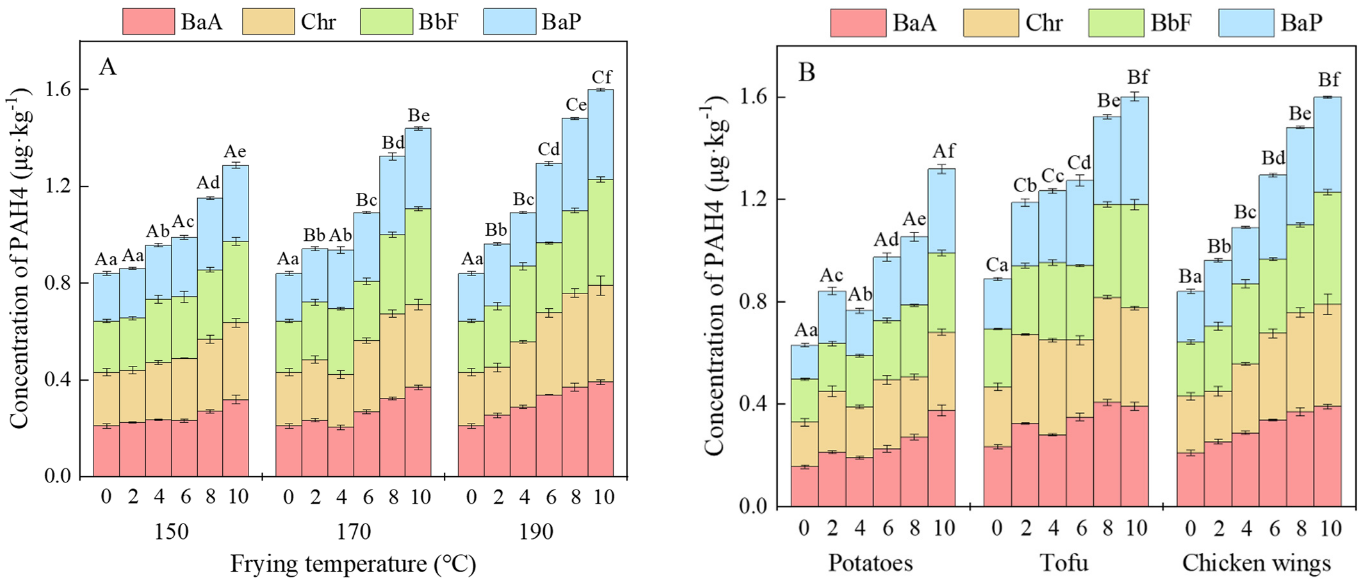

Fig. 4 Changes in PAH4 concentration in chicken wings at different temperatures (A) and in different foods at 190 °C (B) during frying. Different capital letters (A-C) indicate significant differences among the total concentration of PAH4 in different groups within the same heating time ($P < 0.05$). Different lowercase letters (a-f) indicate significant differences among the total concentration of PAH4 in the same groups within different heating time ($P < 0.05$).

The changes in PAH4 concentration in the different raw and fried foods are shown in Fig. 4 (B). The total concentration of PAH4 was 0.63, 0.89, and 0.84 μg·kg^{-1} in raw potatoes, tofu, and chicken wings, respectively. This was consistent with many studies that PAHs accumulated in raw foods from contaminated environments (Lee et al., 2018; Li et al., 2016; Singh & Agarwal, 2018). The PAH4 concentrations in fried potatoes, tofu, and chicken wings at the end of frying were 2.09, 1.80, and 1.90 times higher than that in raw foods, respectively. It can be seen that the concentrations of PAH4 in chicken wings and tofu were significantly higher than that in the potatoes ($P < 0.05$). Generally, PAHs are formed through the incomplete oxidation of organic substances such as fat/oil, proteins, and carbohydrates at high temperatures (Singh et al., 2016). The frying process of potatoes, tofu, and chicken wings meet the conditions for the formation of PAHs. Under identical frying conditions, the high concentration of PAH4 in tofu may be attributed to its high water content. When tofu is fried, the significant loss of water from the tofu provides a pathway for

PAHs to penetrate and remain within the tofu matrix, resulting in higher PAH4 levels compared to other foods.

3.5 Correlation and network relationship among hazards during frying

A heat map (Fig. 5) was constructed to present the Pearson correlations among the food hazards (AA, 5-HMF, HCAs, and PAH4). The correlation coefficients between AA, HCAs, and PAHs in chicken wings at different frying temperatures (150, 170, and 190 °C) were shown in Fig. 5 (A, B, and C). The results showed a consistent trend in the concentrations of AA, total HCAs, and total PAH4 in fried chicken wings with a significant positive correlation ($P < 0.01$). With the increase in frying temperature, the correlation coefficients between HCAs and AA, and PAH4 increased, while the correlation coefficient between AA and PAH4 decreased. It suggested that at higher frying temperatures, HCAs and AA, as well as PAH4 in fried chicken wings, tend to follow a similar trend, whereas AA and PAH4 share a similar trend at lower temperatures (150 °C). Comparing C, D, and E in Fig. 5, the correlation between AA and HCAs was stronger in chicken wings than in potatoes and tofu, possibly related to the rich variety of amino acids in chicken wings that facilitated the formation of AA and HCAs. It is hypothesized that the amino acids in potatoes and tofu may have a substrate competition effect in the formation of AA and HCAs, resulting in lower correlation coefficients. The results of the present study and previous studies proved that both frying temperature and food type affected the correlation between compound types.

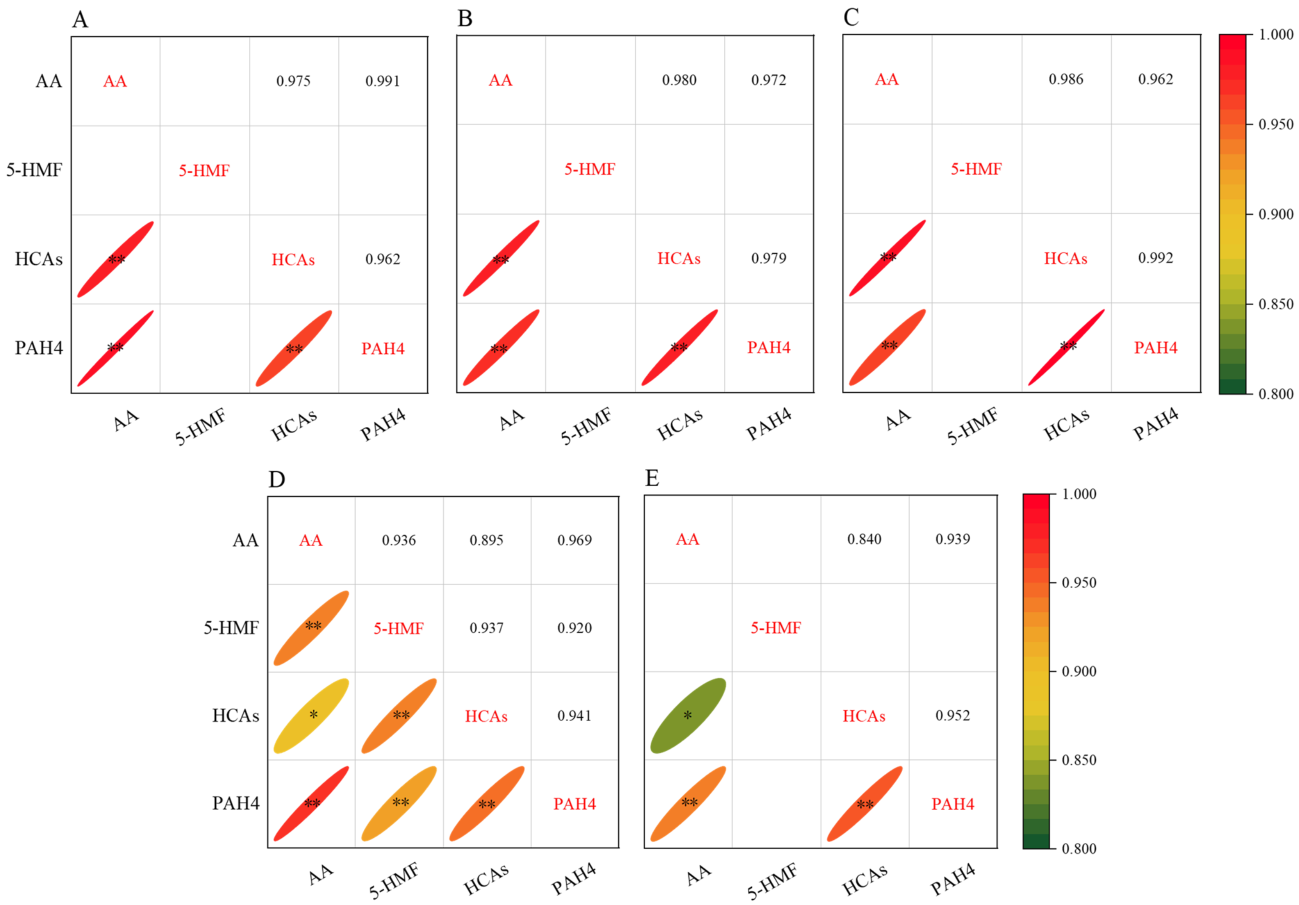

Fig. 5 Heat maps correlation based on the concentration of AA, 5-HMF, total HCAs, and total PAH4 at different temperatures and in different foods at 190 °C during frying. (A) 150 °C; (B) 170 °C; (C) 190 °C; (D) potatoes; (E) tofu. *$p \leq 0.05$; **$p \leq 0.01$.

The network relationship of the main formation pathways of AA, 5-HMF, HCAs, and PAHs is depicted in Fig. 6. Various food basic components provide the necessary conditions for the formation of hazards during frying. AA is mainly formed by the Maillard reaction between amino acid and reducing sugar during thermal processing. Asparagine, a major amino acid in potatoes and cereals, is a crucial participant in the formation of AA via the Asp pathway (Miao et al., 2014). Initially, the nucleophilic amine nitrogen attacks the electrophilic carbonyl carbon of reducing sugar, forming N-glycosyl-asparagine. Schiff's bases form by the interaction between carbonyl and amino groups with the loss of a water molecule (Bachir et al., 2022). Schiff's bases are then cyclized and rearranged to form Amadori compounds, which cleave into carbonyl compounds such as glyoxal. These carbonyl compounds enable amino acids to undergo decarboxylation reactions through the Strecker degradation mechanism to form azomethine ylide. The azomethine ylide hydrolyzes to form 3-aminopropionamide which forms AA through ammonia removal upon heating (Bachir et al., 2022). Furthermore, a minor acrolein pathway for AA formation has been proposed in lipid-rich foods. Triacylglycerol hydrolyzes into glycerol and fatty acids, and acrolein forms through glycerol dehydration or unsaturated fatty acid oxidation (Daniali et al., 2016). Acrolein oxidizes to acrylic acid, which reacts with ammonium from nitrogenous compounds to form AA (Bachir et al., 2022).

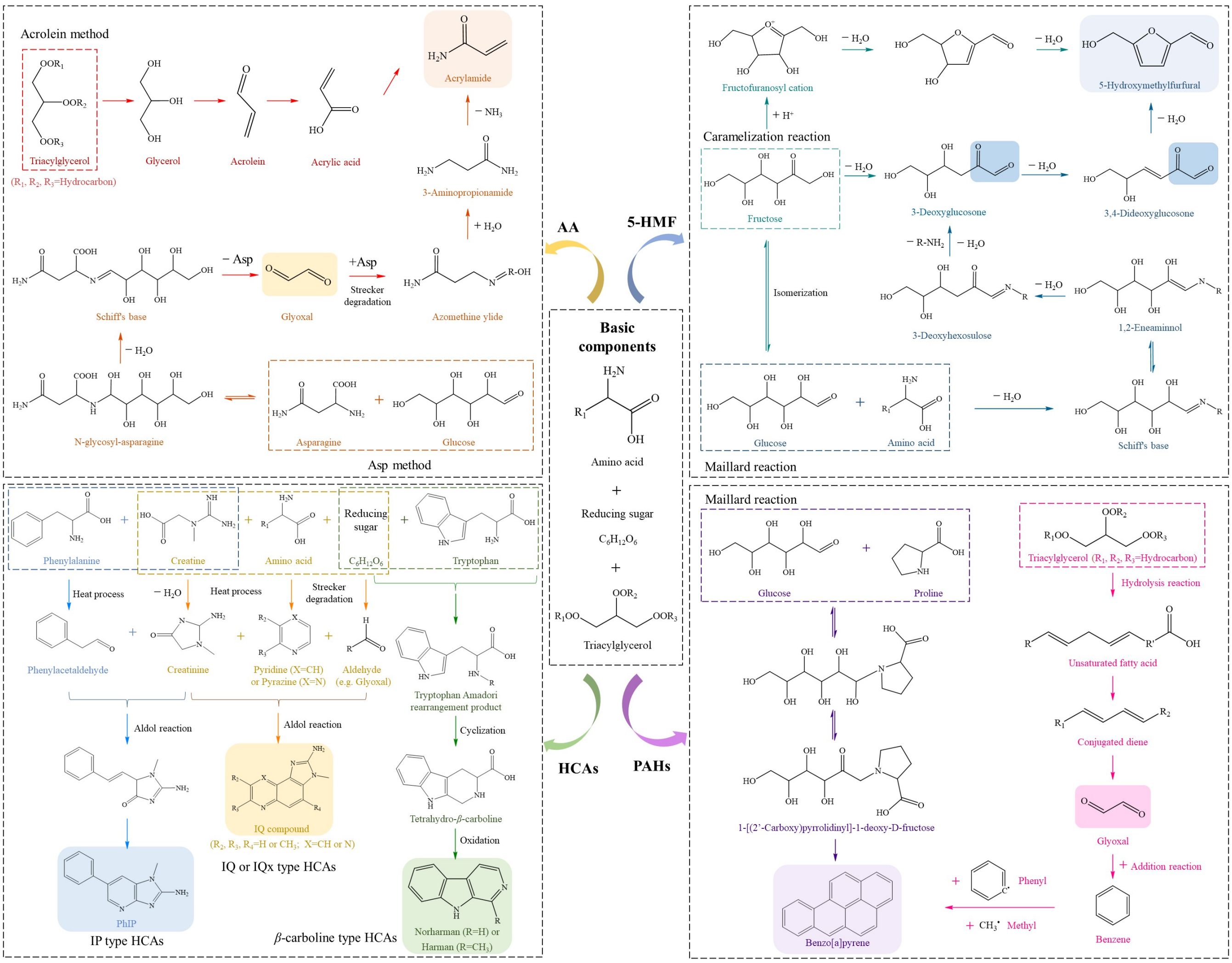

Fig. 6 Network relationship of formation mechanism for AA, 5-HMF, HCAs, and PAHs.

The 5-HMF formation mechanism can be divided into two main pathways: 3-DG decomposition and sugar dehydration under acidic conditions (Martins et al., 2022). Reducing sugars (glucose or fructose) and amino acids form a Schiff's base through the Maillard reaction, which in turn forms Amadori rearrangement products. These compounds are highly unstable and can degrade to 1,2-eneaminnol at pH ≤ 7.0 (Huang et al., 2022). Afterward, dehydration and deamination of 1,2-eneaminnol results in the formation of 3-DG, which is regarded as the key intermediate in 5-HMF formation (Capuano & Fogliano, 2011). Finally, 3-DG forms 3,4-DGE by dehydration yielding 5-HMF through cyclization. Moreover, fructose exposed to thermal treatment forms fructofuranosyl cation through acid-catalyzed degradation, which dehydrates and converts into 5-HMF (Locas & Yaylayan, 2008). Our results revealed a significant positive correlation between 5-HMF and AA in fried potatoes, implying that with increasing 5-HMF concentration, AA concentration also increases, as previous research revealed that HMF was a potent carbonyl to accelerate AA formation during heating (Gökmen et al., 2012).

HCAs can form naturally in protein-rich foods during heating. These HCAs are mainly divided into three types according to their chemical structures: imidazoquinoline or imidazoquinoxaline (IQ or IQx) type, imidazopyridine (IP) type (PhIP), and *β*-carboline type (Harman and Norharman) (Dong et al., 2020). Pyridines, pyrazines, and aldehydes (such as glyoxal) formed from amino acids and reducing sugars, respectively, via Strecker degradation in the Maillard reaction. The creatine cyclizes to creatinine during thermal process and reacts in an aldol reaction with pyridine or pyrazine derivatives, respectively, to form IQ or IQx type HCAs (Gibis, 2016). Regarding the IP type HCAs, the formation mechanism of typical PhIP is well demonstrated. The phenylacetaldehyde from phenylalanine reacts with creatinine to form an aldol condensate, which forms PhIP through the incorporation of formaldehyde and ammonia (Zamora et al., 2014). As representatives of nonpolar HCAs, the formation of *β*-carbolines including Norharman and Harman was widely reported (Li et al., 2023). The tryptophan Amadori rearrangement product is formed from tryptophan with the presence of reducing sugar. It then undergoes cyclization (Pictet-Spengler reaction) to form tetrahydro-*β*-carbolines. Subsequently, the tetrahydro-*β*-carboline undergoes several oxidation steps to form Norharman or Harman (Herraiz, 2000).

The formation mechanisms of PAHs during thermal processes have been explored and proposed possible ways for oil oxidation to form PAHs (Min et al., 2018; Xu et al., 2024). Unsaturated fatty acids, hydrolyzed from triacylglycerols, oxidize to hydroperoxides and undergo intramolecular reactions to form lipid degradation products (with conjugated double bonds). Conjugated dienes oxidize to carbonyl compounds such as glyoxal, which can form benzene through addition reactions. PAHs form by continuous addition/cyclization of low molecular weight cyclic compounds (such as benzene) with intermediate free radicals including phenyl or methyl (Zhu et al., 2022). PAHs formation may also relate to the Maillard reaction. Britt et al. (2004) indicated the equal ratio mixture of proline and glucose forms 1-[(2'-carboxy)pyrrolidinyl]-1-deoxy-D-fructose (a proline Amadori compound) under high temperature, which further pyrolyzes to form PAHs such as BaA, BbF, and BaP (Singh et al., 2016).

Carbohydrates, proteins, and fats are common food components involved in AA, 5-HMF, HCAs, and PAHs formation during thermal treatment due to the Maillard reaction, lipid and protein oxidation, and sugar pyrolysis. Because of the simultaneous formation of these hazards, food component precursors may have competitive effects. Moreover, competitive or synergistic effects may also exist in intermediates of common reactions. Schiff's bases and carbonyl compounds, abundant intermediates in hazard formation, may compete in forming hazards. Schiff's bases, formed by amino group and aldehyde group reaction, play a crucial role in the initial period of hazard formation. Their instability promotes Amadori molecular rearrangement, which results in Amadori products involving the formation of AA, 5-HMF, and partial HCAs. Carbonyl compounds participate as intermediates in the main pathway for the formation of hazards in thermally processed foods, such as 3-aminopropionamide, 3-DG, 3,4-DGE, fructofuranosyl cation, phenylacetaldehyde, creatinine, aldehyde, and 1-[(2'-carboxy)pyrrolidinyl]-1-deoxy-D-fructose. Therefore, glyoxal was selected as a representative intermediate to study its relationship with the concentration of hazards (Fig. 7). The results presented a positive correlation between the concentrations of glyoxal and hazards in food under different frying conditions. As a result, we confirmed that carbonyl compounds are involved in the sugar pyrolysis, Maillard reaction, and lipid oxidation pathways, which promote the formation of hazards during food thermal processing. Furthermore, one of the pathways for the formation of AA and PAHs begins with the hydrolysis of triacylglycerol. The glycerol in the hydrolysis product undergoes oxidation and dehydration to form acrolein, which reacts with ammonia to form AA. The other hydrolysis product, unsaturated fatty acids, can be degraded by oxidation to diene compounds and eventually cyclized to PAHs. This leads to a possible synergistic effect between AA and PAHs formation when triacylglycerol hydrolysis is involved.

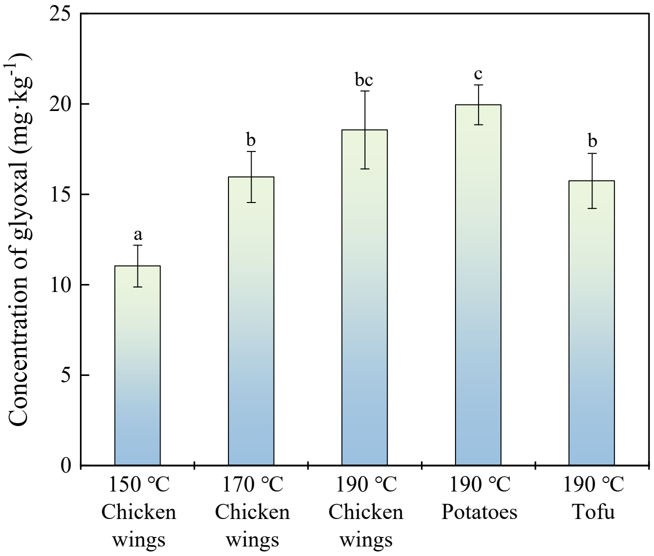

Fig. 7 The concentration of glyoxal in foods after frying in oil heated for 10 h. Different lowercase letters indicate significant differences (P < 0.05).

The formation of AA, 5-HMF, HCAs, and PAHs shares the same reaction precursors, intermediates, and similar reaction pathways, resulting in the interlacement of their formation and developing an intricate network relationship. Foods are put into oil in batches with the process of frying, continuously promoting the release of food component precursors and the formation of intermediates, thus leading to the continuous formation and accumulation of hazards. Therefore, it is necessary to find effective measures to simultaneously reduce multiple hazards in food from the perspective of food components and intermediates to provide a scientific basis for reducing the formation of hazards in the processing process.

4. Conclusions

The study illustrated the formation of food hazards in fried potatoes, tofu, and chicken wings under different frying conditions. AA, HCAs, and PAHs in fried samples and a few 5-HMF in fried potatoes were detectable. Notably, higher frying temperatures and prolonged oil use significantly increased the formation of these hazards. In addition, the concentration of AA and 5-HMF was higher in potatoes with high reducing sugar content, while the concentration of HCAs and PAHs was higher in chicken wings with high fat and protein content. Significant correlations were found between the concentrations of hazards in different fried foods. The network relationship indicated that the formation of AA, 5-HMF, HCAs, and PAHs had the same reaction precursors, intermediates, and similar reaction pathways.

Future research should investigate the interaction mechanisms between different hazards to elucidate their combined effects and develop effective strategies to simultaneously reduce multiple hazards, thus mitigating food safety risks from multiple perspectives. Furthermore, expanding research to include a broader range of food types, edible oil types, and cooking conditions is essential for a comprehensive understanding of the formation and control of food hazards.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Declaration

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Appendix A. Supplementary data

The following supporting information is incorporated in the Supplementary Materials.

Table S1 The abbreviations, chemical formula, molecular weight, chemical structure, and carcinogenic grade of acrylamide, 5-hydroxymethylfurfural, heterocyclic amines, and polycyclic aromatic hydrocarbons.

Table S2 The basic components in raw potatoes, tofu, and chicken wings.

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