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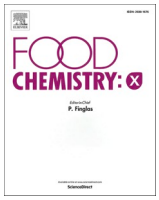

Comparing effects of tangerine-peel (*Citrus reticulata Blanco*) age and concentration on deep-fried rabbit meat: Impact on heterocyclic aromatic amines, amino acids, and flavor compound formation

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ABSTRACT

Many nutritional experts recommend rabbit meat as a high-protein source. However, the high temperatures used to prepare deep-fried rabbit meat (DFRM) typically produce numerous heterocyclic aromatic amines (HAAs), a class of substances with carcinogenic risks. In this study, we chromatographically evaluate changes in the volatile compounds, amino acids, and HAAs in DFRM while employing tangerine peel (TP) as an additive. A total of 35 volatile organic compounds are detected in the TP, which increase the concentrations of sweet and umami amino acids in the DFRM. Remarkably, the TP substantially inhibits the IQ-type HAAs, particularly MeIQ, MeIQx, 4,8-DiMeIQx, and PhIP, which are produced during deep frying. Correlation analyses reveal that the histidine, aldehydes, and alcohols are strongly and positively correlated ($P < 0.001$) with the MeIQ, MeIQx, 4,8-DiMeIQx, and PhIP production. This study offers innovative and natural approaches for reducing HAA formation during the frying of rabbit meat.

1. Introduction

Rabbit meat is globally consumed. In 2020, 70.5 % of the global rabbit meat production quantity originated in Asia (Siddiqui et al., 2023). In particular, the Ira rabbit breed is valued for its rapid growth and high-quality meat. Overall, rabbit meat is low in fat and cholesterol but rich in polyunsaturated fatty acids, proteins, and essential amino acids, thereby offering greater nutritional benefits than other meat sources (Ye et al., 2022).

Flavor is a critical determinant of food purchases and frequently supersedes other influential factors. Frying, a process involving high temperatures, triggers chemical reactions that alter the food molecules. These reactions include Maillard reactions, lipid oxidation, degradation, and Maillard–lipid interactions, which release volatile organic compounds (VOCs) such as aldehydes, ketones, esters, and alcohols. These reactions contribute significantly to the appeal of deep-fried foods for consumers (Auvray & Spence, 2008; Gotow et al., 2013). However, the chemicals present in charred meat (as found in partially to deep-fried foods) include heterocyclic aromatic amines (HAAs) and polycyclic aromatic hydrocarbons, which pose carcinogenic risks. HAAs are a class of chemicals produced when protein-rich foods are subjected to high-temperature cooking methods such as grilling or frying. Robust epidemiological and clinical evidence has associated elevated HAA exposure with an increased risk of various cancers, including those of the colon, prostate, breast, and pancreas. The intricate cellular and molecular mechanisms underlying HAA toxicity encompass DNA adduct formation, oxidative stress, and inflammatory responses (Gibis, 2016). More than 30 HAAs can develop in heated protein-rich foods. In a previous study, the dietary intake of five specific HAAs was calculated: 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,7,8-trimethylimidazo [4,5-*f*]quinoxaline (7,8-DiMeIQx), 2-amino-3,4,8-trimethylimidazo [4,5-*f*]quinoxaline (4,8-DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Iwasaki et al., 2014). The International Agency for Research on Cancer (IARC) of the World Health Organization classifies MeIQ, MeIQx, and PhIP as potential carcinogens (Class 2B). Further, 4,8-DiMeIQx and 7,8-DiMeIQx are potential human carcinogens. Animal studies have shown that these compounds can induce DNA

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damage and tumors in multiple organs (Felton et al., 2006; Sugimura et al., 2004). As a result of their commonality in cooked food and the potential health risks they pose, HAAs have become a significant research focus in the fields of toxicology, food science, and public health.

Certain fruits and spices have been shown to prevent HAA formation in cooked meat products, including mulberry leaves (Xu et al., 2024), blueberries (Gumus and Kizil, 2022a), and basil (Uzun & Oz, 2021). To date, however, the HAA inhibition performance of tangerine peel (TP, *Citrus reticulata Blanco*) has not been investigated. Contemporary research has revealed that TP is rich in polyphenolic compounds, which have medicinal applications (Chen et al., 2022). These compounds function as antioxidants, safeguard cells from free radical damage, and potentially diminish cancer risks by inhibiting tumor formation (Omar et al., 2022). Flavonoids and phenolics are the most prominent groups of these bioactive compounds and act as primary antioxidants and free-radical scavengers (Singh et al., 2020). Notably, the antioxidant scavenging effect on pyrazine cation radicals during HAA generation has been demonstrated using electron spin resonance spectroscopy (Kikugawa et al., 1999).

Considering the potential hazards posed by HAAs to human health, their formation in DFRM must be mitigated. Previous studies have explored the health effects of TP and its protective properties in food. However, the impact of TP on HAA formation remains unexamined, and the effects of the VOCs on HAA inhibition have not been studied.

To bridge this gap, this study investigates the inhibitory effects of TP additive content and aging period (3, 5, 10, and 15 years) on HAA and flavor formation in DFRM. In addition, the relationships among the volatile compounds, HAAs, and amino acids are determined using a Pearson test. The results of this study provide a theoretical basis for improving the flavor and safety of fried rabbit meat.

2. Materials and methods

2.1. Materials and reagents

An Era rabbit (deceased) was purchased from Sichuan Rongwei Agriculture Technology Co., Ltd. (Guangan, China). Premium refined soybean oil(Jinlongyu, Yihaijiali corporation, Shanghai, China) was purchased from a local supermarket in Chengdu, China. Five standard HAAs were purchased from Toronto Research Chemicals Company (Toronto, Canada): MeIQx, 4,8-DiMeIQx, MeIQ, 7,8-DiMeIQx, PhIP, and 2-amino3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline (4,7,8-Tri-MeIQx). Sodium hydroxide, ammonium acetate (purity >98 %), methanol, 2-octanol, ethanol, n-hexane, methylene chloride, acetonitrile, and glacial acetic acid, all of chromatographic grade, were purchased from Shanghai Anpu Experimental Technology Co., Ltd. (Shanghai, China).

2.2. Sample preparation

An Era rabbit (deceased), approximately 1 year old and weighing 5 kg, served as the meat source. Following sacrifice, the carcass was immediately transported to the laboratory in an insulated container, which maintained a temperature below 4 °C. The transit time did not exceed 1 h. Upon arrival, the meat was promptly divided into experimental portions of approximately 100 g each. These portions were vacuum sealed in food-grade plastic bags to prevent freezer burning and contamination. The samples were then rapidly frozen to −18 °C using a blast freezer and stored at this temperature for a maximum of 15 d before use. Prior to experimentation, the meat samples were thawed under controlled conditions in a refrigerator at 4 °C for 24 h to ensure uniform and gradual thawing; hence, any potential for microbial growth or meat-quality degradation was minimized.

TP samples aged 3, 5, 10, and 15 years were soaked in deionized water (TP-to-water ratio: 1:40) at ambient temperature for 2 h and then pulverized into a slurry. The rabbit longissimus lumbar muscle was portioned into 70-g meat portions measuring 2.0 × 2.0 × 1.5 cm. The soybean oil was heated to 240 ± 1 °C and the meat portions were fried for 2 mins, with an oil temperature of 160 ± 1 °C being measured post-cooking. TP slurries composed of the TP samples of various ages were then mixed with 50 g of the rabbit meat in proportions corresponding to approximately 0 %, 2 %, 4 %, and 6 % of TP dry mass (*w*/w). The samples were labeled 3 N-2, 3 N-4, 3 N-6, 5 N-2, 5 N-4, 5 N-6, 10 N-2, 10 N-4, 10 N-6, 15 N-2, 15 N-4, and 15 N-6, where the first and second numbers in each label indicate the TP age and dry-mass proportion, respectively. The samples were vacuum-sealed and stored at −80 °C before analysis.

2.3. DFRM flavor detection using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS)

The volatile organic flavor compounds of the samples were determined according to the method described by Wu et al. (2019), with slight modifications. Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS) was performed. The HS-SPME parameters were as follows. A solid-phase microextraction device (50/30 μm DVB/CAR/PDMS, Supelco, USA) was preconditioned at a gas chromatograph inlet for 30 mins at 250 °C before use. Upon preconditioning, a 0.50-g sample was precisely placed into a 15-ml headspace vial. Subsequently, 0.01 g of 2-octanol, which served as the internal standard, was added. The SPME equipment then facilitated extraction at a stable temperature of 50 °C for a duration of 20 mins. This was followed by a 5-min desorption period at the GC–MS inlet prior to automation of the injection process.

The GC parameters were as follows. A GC–MS (7890B-5977 A, Agilent Company, USA) equipped with a 60 m × 0.25 mm × 0.25 μm DB-WAX capillary column (J&W Company, USA) was employed. Helium (99.999 %) was used as the carrier gas. The temperature programming was initiated at 40 °C. This temperature was maintained for 3 mins and then ramped to 230 °C at a rate of 3 °C/min. This temperature was maintained for 10 mins, which was followed by a 3 min post-run. The constant flow rate was 1.0 mL/min, the inlet temperature was 250 °C, and the split ratio was 5:1. The MS parameters were as follows. The mass spectrometer was operated with an electron-impact ion source at 70 eV. The transfer line temperature was set to 280 °C, the ion source temperature was 230 °C, and the quadrupole temperature was 150 °C. The mass scanning range was from 30 to 550 (*m/z*), and the sampling interval was set to 0.3 s with a scanning rate of 1666 amu/s. Each sample was tested in triplicate to ensure result stability. The volatile compounds were identified by comparing the experimental mass spectra with the National Institute of Standards and Technology 17 mass spectral library, with 90 % as the minimum degree of similarity.

2.4. Amino-acid detection

The amino-acid were determined according to the method described by Xu et al. (2023), with slight modifications. An automatic amino acid analyzer (L-8800, Hitachi Instruments Engineering, Tokyo, Japan) was used to assess the amino acid composition of the fried meat. For precise measurement, 0.150 g of each sample was dissolved in 10 mL of 6-mol/L hydrochloric acid in a sealed test tube. The tube was then subjected to hydrolysis in a sand bath maintained at 110 ± 1 °C for 22 h. After hydrolysis, the sample was cooled to ambient temperature, shaken vigorously, and filtered. Next, a 1-mL aliquot of the filtrate was transferred to a 50-mL beaker and evaporated to dryness in a water bath set at 60 °C. Then, 4 mL of 0.02-mol/L hydrochloric acid was added, and the solution was filtered through a 0.22-μm organic filter membrane for amino acid analysis. The analysis was performed using a 4.6 mm × 60 mm ion exchange resin-packed column (855–3530, 5-μm particle size). The analysis cycle for each sample lasted 53 min with an eluent flow rate of 0.4 mL/min.

2.5. HAA determination

The HAAs were extracted and quantified following the method described by Xu et al. (2023), with slight modifications. The linear range of the mixed sample standard curve for HAA determination was 10–100 μL/kg. The HAAs were detected using ultra-high-performance liquid chromatography (HPLC)–triple quadrupole tandem mass spectrometry (Sciex 3500, USA). A 2.00-g portion of each sample was weighed (accurate to 0.01 g) and placed in a 50-mL centrifuge tube, Then, 200 μL of internal standard working solution (4,7,8-TriMeIQx) and 9.8 mL of 1-mol/L sodium hydroxide-methanol (7:3, *v*/v) were added in sequence and homogenized (IKAT-18, Germany). Then, 5 mL of 1-mol/L sodium hydroxide–methanol was added to the sample and extraction was performed twice. The extracts were combined in a centrifuge tube at 8000 rpm for 5 min (Sigma 3 K15, Sigma Company, USA). The cartridge was activated with 2 mL of methanol, followed by distilled water and 0.1 mol/L of hydrochloric acid (HCl). The column was then flushed with 3 mL of 0.1-mol/L HCl and methanol, and 6 mL of methanol–ammonia (9:1, v/v) solution was passed through. The eluate was collected and dried under a nitrogen atmosphere. The dried eluate was redissolved in 200 μL of methanol, mixed thoroughly, and then passed through a 0.22-μm organic filter for HPLC analysis. For samples exceeding the upper limit of the standard curve (100 μL/kg), appropriate dilutions were made with methanol prior to analysis to ensure measurements fell within the linear range of 10–100 μL/kg.

Liquid-phase parameters: An Agilent Eclipse Plus C18 column (100 × 2.1 mm, 1.8 um) was used for separation. The mobile-phase flow rate was 0.3 mL/min. The gradient elution conditions were as follows: mobile phase A: 30-mol/L ammonium acetate (pH 3.5); mobile phase B: acetonitrile. The following gradient elution program was employed: 90 % A at 0–0.5 min, 90–40 % A at 0.5–5.0 min, 40–5 % A at 5.0–6.0 min; 5 % A at 6.0–8.0 min; 5–90 % A at 8.0–8.1 min; and 90 % A at 8.1–9.0 min. In this process, the next injection was initiated after a 5-min equilibration period. The mobile phase flow rate was 0.3 mL/min and the column and sample temperatures were 40 and 15 °C, respectively. An autosampler was configured to inject a 2-μL volume of each sample for analysis. To maintain sample integrity and ensure consistent chromatographic performance, the autosampler temperature was maintained at 5 °C throughout the analysis. The MS conditions were as follows: an electrospray ion source and positive ionization mode (electrospray ionization, ESI) were used. The specific ESI parameters were as follows: capillary voltage: 3.0 kV, ion source temperature: 100 °C, desolvation gas temperature: 350 °C, gas flow rate: 800 L/h (nitrogen), cone gas flow rate: 50 L/h (nitrogen), and detection mode: multiple reaction detection.

2.6. Statistical analysis

All experiments were performed in triplicate ($n = 3$). The data are presented as mean ± standard deviation (SD) values. Tukey 's multiple comparison test was used for an analysis of variance to evaluate the significance of the treatment effects. This test was performed using SPSS software (version 25.0; IBM Corporation, Armonk, NY, USA) with a significance level of $P < 0.05$. Chemical structure diagrams of the identified VOCs were plotted using ChemDraw 20.0 (PerkinElmer, Waltham, MA, USA). All figures were plotted using Origin 2022 software (Origin Lab Corporation, Northampton, MA, USA). The Pearson's correlation was analyzed using Chiplot software.

3. Results and discussion

3.1. Analysis of volatile organic compounds (VOCs)

In this study, we detected 35 VOCs in the TP, comprising 14 alkenes, six aldehydes, five alcohols, five esters, two ketones, two benzenes, and one phenol. Fig. 1(a) illustrates the VOC distribution across the TP samples aged 3, 5, 10, and 15 years. The 3-year TP exhibited the highest VOC diversity, followed by the 10- and 5-year TP, whereas the 15-year TP had the lowest diversity. Alkenes, aldehydes, alcohols, and esters were the predominant VOCs in all aged TP samples. Fig. 1(b) presents the total VOC content of each TP type; the results mirror the trend observed for the VOC diversity. The 3-year TP had the highest content, whereas the 5-year sample showed a significant decrease ($P < 0.01$) and the 15-year TP exhibited an extremely significant reduction ($P < 0.001$). Note that the 10-year TP volatile matter content also declined, but with low significance ($P < 0.05$). The esters and aldehydes almost vanished and the phenols were completely degraded of 10-year TP. The total VOC losses were greater in the 5- and 15-year TP than in the 10-year TP.

The TP aroma biosynthesis was categorized into the terpenoid, fatty-acid, and amino-acid metabolic pathways based on the substrate types involved. Note that terpenes in fruit are primarily produced via the terpenoid pathway. Fatty acids serve as precursors in the fatty acid pathway, causing the formation of most alcohols, aldehydes, ketones, and esters. Conversely, branched-chain aliphatic alcohols, aldehydes, ketones, and esters are mainly synthesized via the amino acid pathway. The interplay between different substrates and enzymatic activities culminates in distinct aromatic compounds, which ultimately define the TP aroma.

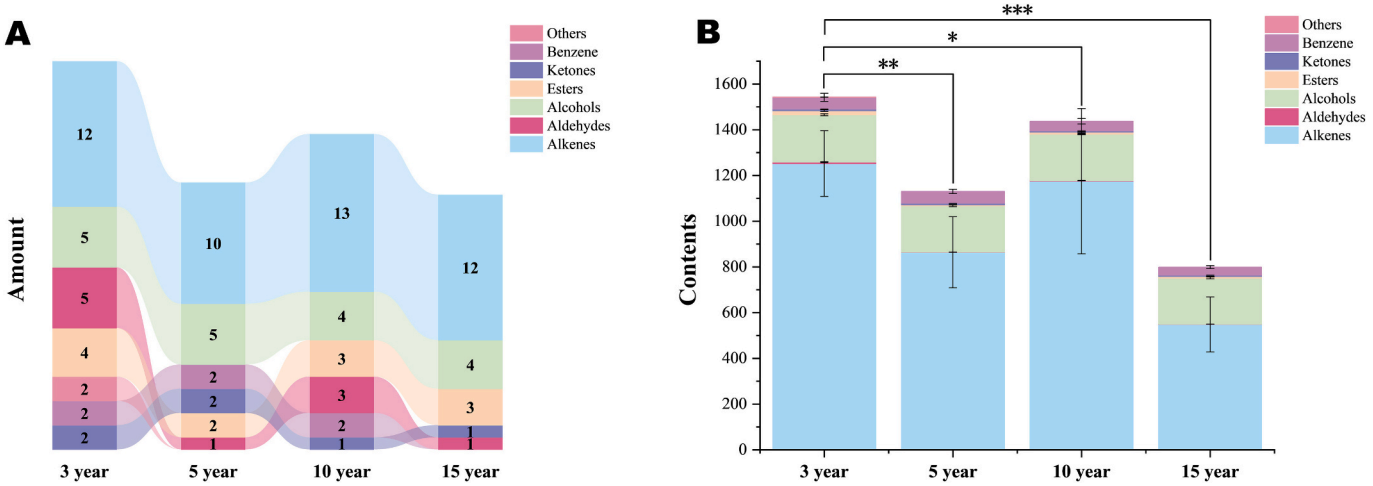


Fig. 1. Volatile organic compound (VOC) concentrations and types in differently aged tangerine peel (TP) samples: **(a)** Classification and **(b)** quantification of VOCs in TP. *, **, and *** indicate significant differences at the level of $P < 0.05$, < 0.01, and < 0.001, respectively.

Fig. 2 shows the VOCs with concentrations exceeding 1 μg/kg. The alkenes were most prevalent within the TP. Alkenes predominantly arise from oxidative degradation of lipids, which is typically observed during fermentation and drying phases. Alkenes in TP are characterized by low threshold values and contribute significantly to the overall flavor profile (Li et al., 2023). Table 1 reports the marked variations in VOC content across the differently aged TP samples, with a statistical significance of $P < 0.001$. In particular, D-limonene, which imparts a sweet, citrusy, and lemony essence, exhibited an exceptionally low threshold value of 1.2 μg/kg. Its presence in the TP was akin to that for oranges, lemons, and essential oils. The D-limonene acted as a quintessential marker of the TP characteristic aroma, consistent with the findings of previous studies (Li, Lin, et al., 2022). Furthermore, D-limonene is known for its multifaceted pharmacological properties, including anti-inflammatory, antioxidant, and antidepressant effects (Dambolena et al., 2008; Tang et al., 2022).

The concentrations of the alkene compounds, particularly D-limonene, decreased significantly with aging, indicating a pronounced negative correlation. The D-limonene content in the 3-year TP was 953.87. This value diminished by 30.91 % after five years and plummeted by approximately 57.40 % after 15. However, a notable deviation was observed for the 10-year TP, as the alkene decrease slowed and the overall content surpassed that of the 5-year TP. This may have been due to the time, temperature, and humidity during the drying process, which may have influenced the microbial ecosystem within the TP, thereby altering its chemical composition (d'Alessio et al., 2022). Such environmental conditions can affect the rate of oxidative lipid degradation, which is the primary pathway for alkene production during fermentation and drying (Yue et al., 2023). The intricate interplay between these variables and the microbial flora may explain the observed variations in alkene concentrations across different aging periods.

The results indicated that γ-terpinene ranked second in terms of total content; this substance is distinguished by its lemon scent and citrus flavor. Having a threshold of 10.1 μg/kg, it is a primary flavor contributor of TP. Research indicates that γ-terpinene possesses antitumor and antioxidant properties, rendering it pharmacologically relevant (Salehi et al., 2019). The transition trend of γ-terpinene paralleled that of D-limonene, peaking at 179.52 in the 3-year TP, decreasing by 53.53 % in the 15-year TP, and rising by 7.34 % in the 10-year TP (to 192.71). Myrcene and α-pinene emit subtle citrus and pine notes, contributing to the flavor harmony of TP. Their concentrations in the 3-year TP were 44.11 and 28.56, respectively, and significantly lower in the 15-year TP, by approximately 57 % and 42 %, respectively. However, for the 10-year TP, their levels remained mostly unchanged. Alkenes such as β-pinene, terpinolene, α-thujene, and α-terpinen are prevalent in citrus fruits and contribute to the flavor and medicinal value of TP (Menezes et al., 2021). These compounds were present in higher levels in the 10-year TP compared to the 3-year sample. Moreover, α-farnesene was present in minimal amounts in the 3- and 15-year TP, whereas β-ocimene, with has an orange aroma, was detected in the 3- and 10-year TP only. α-Copaene emerged as a unique compound in the 10-year TP, and sabinene was found in the 10- and 15-year TP samples. These volatile-compound fluctuations may be attributed to metabolic transformations of the precursor substances, along with isomerization and decomposition interactions among the components (Hu et al., 2019).

Despite their high thresholds, alcohols impart floral, fruity, and citrus aromas to TP. Most alcohol content declines with aging, likely because of enzyme inactivation and loss of aromatic precursors during drying (Downs & Johnson, 2018). Among the alcohols, 2-octanol was dominant in the TP, constituting 96.6 % of the total alcohol content and

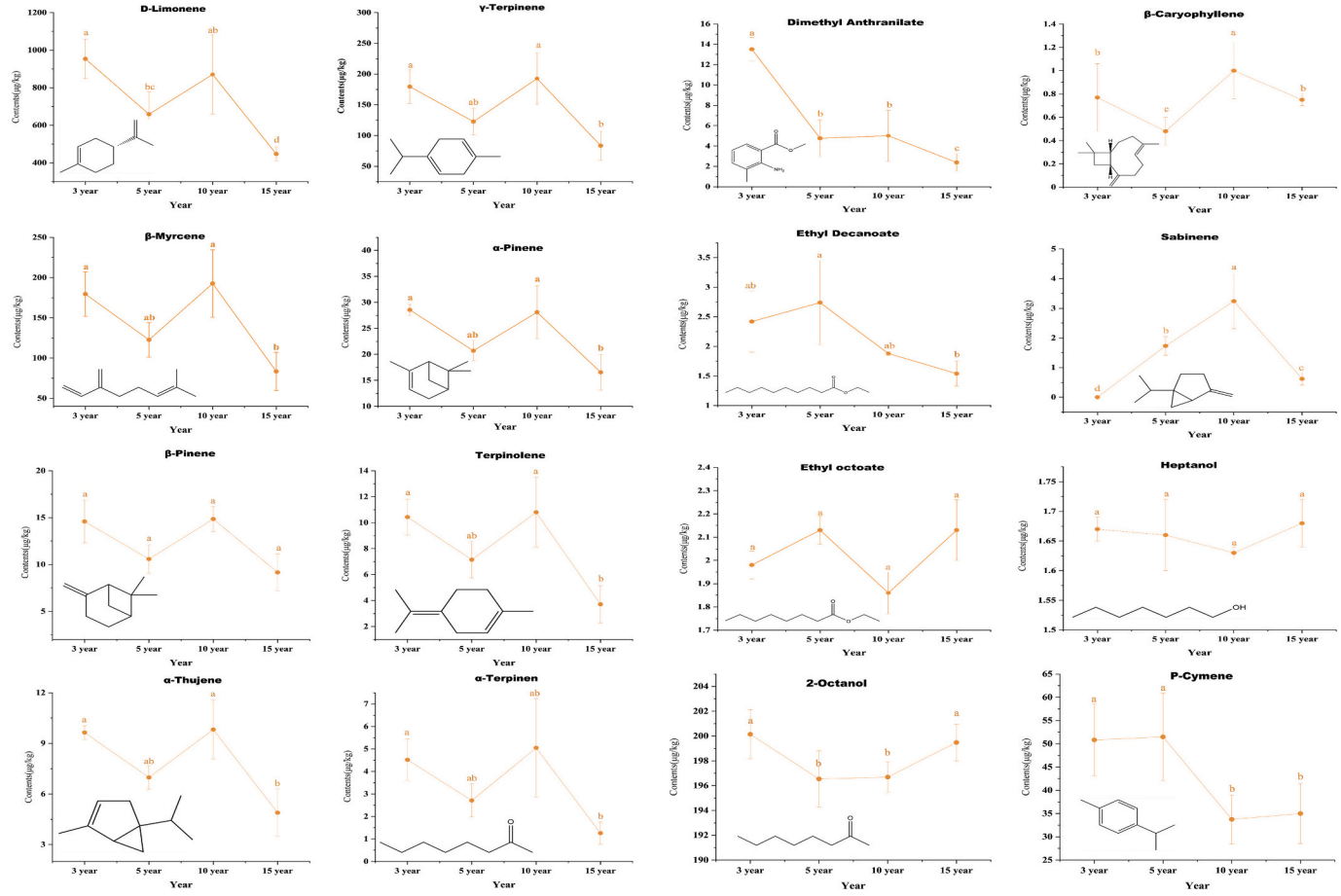
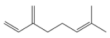
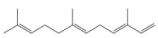
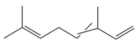
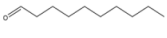
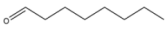
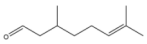
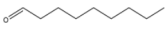
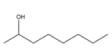
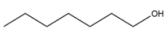
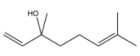


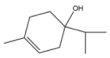
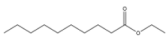
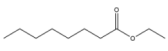
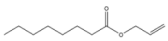
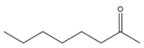
Fig. 2. Predominant VOC concentrations in TP samples aged 3, 5, 10, and 15 years. The error bars with different letters indicate significant differences ($P < 0.05$).

Table 1
Analysis of volatile organic compounds in tangerine peel aged 3, 5, 10, and 15 years.

Count	Classification	Compound name	CAS#	Formula	Structural type	Molecular weight	Retention time	Contents			
								3 year	5 year	10 year	15 year
1	Alkenes	D-Limonene	5989-27-5	C10H16		136.13	18.95	953.87 ± 104.76^{a}	658.98 ± 120.95^{bc}	870.54 ± 210.06^{ab}	447.27 ± 18.15^{d}
2		γ-Terpinene	99–85-4	C10H16		136.13	21.05	179.52 ± 27.62^{a}	122.67 ± 21.58^{ab}	192.71 ± 71.88^{a}	83.39 ± 23.70^{b}
3		β-Myrcene	123–35-3	C10H16		136.13	17.17	44.11 ± 3.53^{a}	30.80 ± 5.85^{c}	33.75 ± 5.25^{b}	19.15 ± 4.75^{d}
4		α-Pinene	80–56-8	C10H16		136.13	11.31	28.56 ± 1.02^{a}	20.70 ± 1.97^{ab}	28.11 ± 10.07^{a}	16.55 ± 3.43^{b}
5		β-Pinene	127–91-3	C10H16		136.13	14.58	14.59 ± 2.29^{a}	10.58 ± 1.51^{a}	14.85 ± 1.32^{a}	9.15 ± 1.99^{a}
6		Terpinolene	586–62-9	C10H16		136.13	22.75	10.43 ± 1.39^{a}	7.15 ± 1.42^{ab}	10.80 ± 2.68^{a}	3.71 ± 1.44^{b}
7		α-Thujene	2867-05-2	C10H16		136.13	11.49	9.64 ± 0.41^{a}	6.98 ± 0.70^{ab}	9.82 ± 1.75^{a}	4.89 ± 1.41^{b}
8		α-Terpinen	99–86-5	C10H16		136.13	17.91	4.52 ± 0.92^{a}	2.71 ± 0.74^{ab}	5.05 ± 2.19^{a}	1.26 ± 0.49^{b}
9		β-Phellandrene	555–10-2	C10H16		136.13	14.58	2.45 ± 0.43^{b}	2.74 ± 0.56^{ab}	3.97 ± 1.15^{a}	1.72 ± 0.37^{b}
10		α-Farnesene	502–61-4	C15H24		204.19	42.02	1.16 ± 1.01^{b}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
11		β-Caryophyllene	87–44-5	C15H24		204.19	36.48	0.77 ± 0.29^{b}	0.48 ± 0.12^{c}	1.00 ± 0.24^{a}	0.75 ± 0.05^{b}
12		β-Ocimene	13,877–91-3	C10H16		136.13	21.24	0.73 ± 0.04^{a}	0.00 ± 0.00	0.73 ± 0.12^{a}	0.00 ± 0.00
13		α-Copaene	3856-25-5	C15H24		128.12	22.81	0.00 ± 0.00	0.00 ± 0.00	0.68 ± 0.09	0.00 ± 0.00
14		Sabinene	3387–41–5	C10H16		136.13	14.94	0.00 ± 0.00^{d}	1.73 ± 0.31^{b}	3.24 ± 0.93^{a}	0.62 ± 0.21^{c}
15	Aldehydes	Decanal	112–31-2	C10H20O		156.15	32.25	1.72 ± 0.83^{a}	0.77 ± 0.35^{a}	1.30 ± 0.42^{a}	0.00 ± 0.00
16		Octanal	124–13-0	C8H16O		128.12	23.02	1.34 ± 0.26	0.00 ± 0.00	0.56 ± 0.09	0.00 ± 0.00
17		Perilla aldehyde	2111-75-3	C10H14O		150.1	43.62	1.34 ± 0.55	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18		Citronellal	106–23-0	C10H18O		154.14	31.41	0.83 ± 0.08	0.00 ± 0.00	0.75 ± 0.34	0.00 ± 0.00
19		Nonanal	124–19-6	C9H18O		142.14	27.72	0.79 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20		furfural	98–01-1	C5H4O2		96.021	30.70	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.41 ± 0.05
21	Alcohols	2-Octanol	123–96-6	C8H18O		130.14	28.98	200.14 ± 1.97^{a}	196.54 ± 2.27^{b}	196.69 ± 1.23^{b}	199.48 ± 3.77^{a}
22		α-Terpineol	98–55-5	C10H18O		154.14	40.29	2.93 ± 1.20^{b}	2.71 ± 0.74^{c}	3.23 ± 0.12^{a}	0.48 ± 0.07^{d}
23		Heptanol	111–70-6	C7H16O		116.12	30.48	1.67 ± 0.02^{a}	1.66 ± 0.06^{a}	1.63 ± 0.01^{a}	1.68 ± 0.04^{a}
24		Linalool	78–70-6	C10H18O		154.14	34.23	1.30 ± 0.37^{a}	0.61 ± 0.11^{b}	1.17 ± 0.14^{a}	0.00 ± 0.00^{c}

(*continued on next page*)

Table 1 (*continued*)

Count	Classification	Compound name	CAS#	Formula	Structural type	Molecular weight	Retention time	Contents			
								3 year	5 year	10 year	15 year
25		4-Terpinenol	562–74-3	C10H18O		154.14	36.64	1.11 ± 0.07^{ab}	0.88 ± 0.09^{bc}	1.42 ± 0.11^{a}	0.60 ± 0.11^{c}
26		(−)-cis-Carveol	1197-06-4	C10H16O		152.12	45.33	0.53 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
27	Esters	Dimethyl Anthranilate	22,223–49-0	C9H11NO2		165.08	53.46	13.51 ± 1.15^{a}	4.76 ± 1.88^{b}	5.01 ± 2.50^{b}	2.38 ± 0.84^{c}
28		Ethyl Decanoate	110–38-3	C12H24O2		200.18	37.90	2.42 ± 0.52^{ab}	2.74 ± 0.71^{a}	1.88 ± 0.03^{ab}	1.54 ± 0.21^{b}
29		Ethyl octoate	106–32-1	C10H20O2		172.15	29.54	1.98 ± 0.06^{a}	2.13 ± 0.06^{a}	1.86 ± 0.09^{a}	2.13 ± 0.13^{a}
30		allyl caprylate	4230-97-1	C11H20O2		184.27	28.70	0.52 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
31	Ketones	2-Octanone	111–13-7	C8H16O		128.12	22.87	5.66 ± 0.05^{b}	5.67 ± 0.06^{b}	5.78 ± 0.11^{b}	6.06 ± 0.14^{a}
32		D-Carvone	2244-16-8	C10H14O		150.10	41.77	0.61 ± 0.11	0.50 ± 0.09	0.00 ± 0.00	0.00 ± 0.00
33	Benzenes	P-Cymene	99–87-6	C10H14		134.11	22.15	50.82 ± 7.75^{a}	51.48 ± 9.40^{a}	33.75 ± 5.25^{b}	35.00 ± 6.43^{b}
34		P-Cymenyl	1195-32-0	C10H12		132.09	29.62	0.64 ± 0.08^{a}	0.53 ± 0.04^{a}	0.57 ± 0.05^{a}	0.00 ± 0.00
35	Phenol	Thymol	89–83-8	C10H14O		150.10	56.67	0.89 ± 0.62	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

imparting citrus and grassy notes. Its concentration decreased marginally by approximately 3.6 μg/kg in the 5- and 10-year TP and by 0.66 μg/kg in the 15-year TP, being the smallest change. α-Terpineol enhances the floral aroma of TP; in this study, a significant decrease in α-terpineol of 83.62 % was observed in the 15-year TP; however, the content increased in the 10-year TP. Heptanol and linalool contribute spicy, floral, and citrus-like aromas; here, the content of the former decreased slightly with age, whereas the latter, along with 4-terpinenol, was absent from the 10- and 15-year TP samples.

Aldehydes, which are known for their low thresholds, are critical flavor components. Here, a total of six aldehydes were detected, with five in the 3-year TP, three in the 10-year TP, and only one in both the 3-year and 15-year TP samples. However, their contents and variety diminished markedly over time, particularly in the 5- and 15-year TP, where the variety contracted from five to a single substance. Citronellal and nonanal, which impart spicy, lipid, and citrus aromas, were exclusively identified in the 3-year TP. These compounds are pivotal as regards TP quality grading. Octanal and citronellal were detected in the 3- and 10-year TP samples only, with a notable decrease in the older sample. The content of the esters, which typically impart sweet and fruity notes to TP, initially increased and then decreased. Their contribution to the general TP aroma depended on the ester chain length. As regards ethyl decanoate and ethyl octoate, which have short chains, their concentrations were highest in the 3-year TP but declined in the 10- and 15-year samples. This trend may be attributed to the effects of hydration reactions during aging on the ester compounds, which caused their hydrolysis into alcohols, phenols, and carboxylic compounds (Zhang et al., 2016). The concentration of 2-octanone, which is known for its floral scent, increased with aging. Conversely, d-carvone was absent in the younger TP sample, but emerged in those aged 10 and 15 years. p-Cymene, which had a low threshold of 5.01 μg/kg, imparts a robust citrus flavor to TP. Its content peaked in the 5-year TP but declined by 30 % in the 15-year TP, being undetectable in the latter.

Overall, the aging process significantly influenced the TP evaluation results, with each stage revealing a unique VOC profile critical for assessing the sample quality and age. In particular, D-limonene can serve as a key quality indicator, whereas compounds such as allyl caprylate, thymol, and (−)-cis-Carveol are exclusive to 3-year TP, and furfural is unique to 15-year TP. Careful tracking of these compounds is essential for precise TP grading, which helps prevent market fraud and protects consumers from financial harm.

3.2. Analysis of amino acids

Diversity in the types and quantities of free amino acids in different products contribute to their unique flavors. Further, free amino acids are pivotal indicators of food quality and precursors of VOCs and HAAs (Yang et al., 2024). In this study, the amino-acid types and concentrations in the TP were determined (Table 2 and Fig. 3a). Proline (Pro), which provides TP with a sweet taste, was the most abundant amino acid; its contents at 3, 5, and 10 years were similar, but that at 15 years was the lowest. Cysteine (Cys) and aspartic acid (Asp), which have sweet and umami flavors, respectively, impart sweet and fresh flavors to TP. These amino acids exhibited similar concentration trends to that of Pro. Overall, prolonged aging appeared to destroy the original amino acids. The 5-year TP had the highest total free amino acid content; in contrast, the 3-year TP had the lowest total amino acid content.

Table 3 and Fig. 3(b) show the concentrations after the fried rabbit was mixed with the TP. Analysis of specific amino acids at concentrations exceeding 10 in various samples revealed that serine (Ser, sweet), arginine (Arg, sweet), glycine (Gly, sweet), glutamic acid (Glu, umami), alanine (Ala, sweet), and lysine (Lys, bitter) were predominant in the control group, providing umami or sweet flavors. Previous research has indicated that overall consumer satisfaction is significantly enhanced for samples abundant in umami and sweet flavors, with greater contentment and relaxation, as well as positive emotions such as joy and

Table 2
Amino acid concentration in Tangerine peel (mg/100 g) (n = 3).

Amino acids	Flavor characteristics	Molecular weight	3 year	5 year	10 year	15 year
Proline (Pro)	Sweet	115.13	2.23 ± 0.87	2.43 ± 1.02	2.41 ± 0.88	1.33 ± 0.49
Cystine (Cys)	Sweet	240.02	0.52 ± 0.90	1.45 ± 0.54	1.01 ± 0.11	0.61 ± 0.06
Aspartic acid (Asp)	Umami	133.10	0.43 ± 0.29	1.20 ± 0.66	0.88 ± 0.17	0.26 ± 0.09
Alanine (Ala)	Sweet	89.09	0.32 ± 0.06	0.25 ± 0.13	0.26 ± 0.06	0.14 ± 0.05
Tyrosine (Tyr)	Bitter	181.19	0.23 ± 0.06	0.15 ± 0.05	0.14 ± 0.03	0.13 ± 0.02
Serine (Ser)	Sweet	105.09	0.16 ± 0.05	0.13 ± 0.07	0.14 ± 0.01	0.07 ± 0.00
Leucine (Leu)	Bitter	131.17	0.13 ± 0.01	0.12 ± 0.03	0.11 ± 0.05	0.06 ± 0.01
Methionine (Met)	Bitter	149.21	0.13 ± 0.05	0.11 ± 0.03	0.11 ± 0.06	0.05 ± 0.01
Arginine (Arg)	Sweet	174.20	0.11 ± 0.03	0.11 ± 0.01	0.10 ± 0.01	0.03 ± 0.01
Phenylalanine (Phe)	Bitter	165.19	0.11 ± 0.01	0.10 ± 0.08	0.09 ± 0.01	0.02 ± 0.02
Isoleucine (Ile)	Bitter	131.17	0.09 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.02 ± 0.01
Valine (Val)	Bitter	117.15	0.05 ± 0.02	0.07 ± 0.02	0.05 ± 0.03	0.01 ± 0.01
Lysine (Lys)	Bitter	146.19	0.02 ± 0.03	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Glycine (Gly)	Sweet	75.07	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Glutamic acid (Glu)	Umami	147.13	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Histidine (His)	Bitter	155.16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Threonine (Thr)	Sweet	119.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total Free Amino Acid Content			4.55	6.21	5.38	2.74
Total essential amino acids (EAA)			0.67	0.55	0.51	0.28

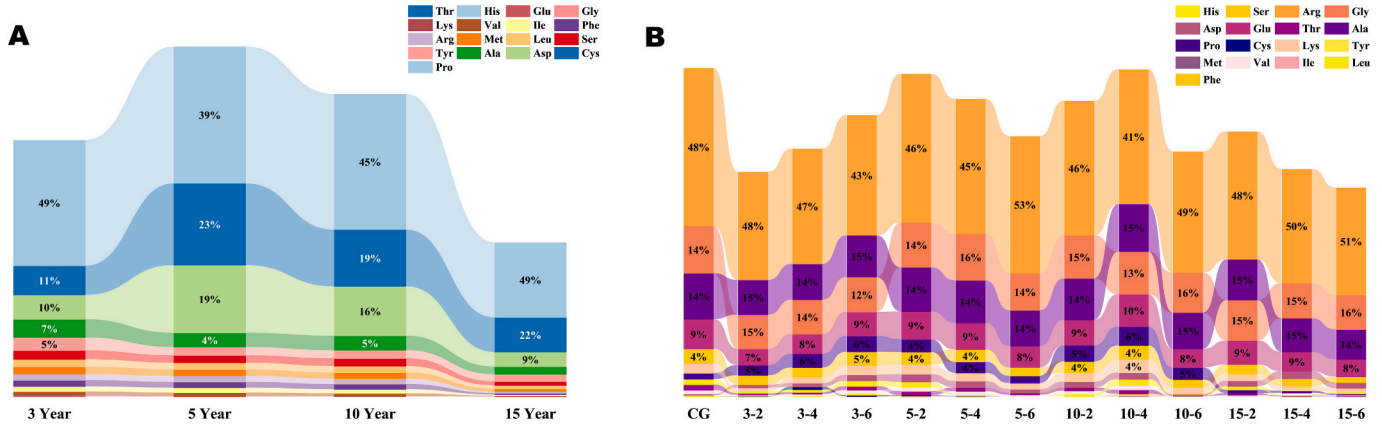


Fig. 3. Amino acid concentrations in aged TP and fried rabbit meat with TP. Amino acid concentrations in **(a)** TP samples aged 3, 5, 10, and 15 years and **(b)** DFRM with various TP ages and additive amounts. CG: Control group. 3 N-2, 3 N-4, and 3 N-6: 2, 4, and 6 % 3-year TP, respectively; 5 N-2, 5 N-4, 5 N-6: 2, 4, and 6 % 5-year TP, respectively; 10 N-2, 10 N-4, 10 N-6: 2, 4, and 6 % 10-year TP, respectively; and 15 N-2, 15 N-4, and 15 N-6: 2, 4, and 6 % 15-year TP, respectively.

excitement, being reported (Desmet & Schifferstein, 2008; Miyaki et al., 2016). This enhancement may contribute to the popularity of DFRM among consumers. Following TP incorporation, changes in the concentrations of these particular amino acids were observed. Specifically, the TP age and additive amount adversely affected the Ser, Arg, and Gly levels. These amino acids, and particularly Arg, exhibited consistent concentration declines with increasing TP age and additive amount. The Glu and Ala concentrations also decreased, with the exception of the 10 N-4 sample. Conversely, marginal increases in Lys content were observed for the 5 N-2, 5 N-4, and 10 N-4 samples; decreasing trends were otherwise observed. Pro, which is characterized by its sweet taste, was quantified at 9.08 in the control group. Following TP addition, significant fluctuations in Pro content were observed across the samples. For the 3-year TP, the Pro levels increased proportionally with the TP additive amount. In contrast, reductions in Pro content were observed for the 5- and 15-year TP samples with higher TP additive amounts. For the 10-year TP, the Pro concentrations peaked in the 10 N-4 sample before declining. Cys (sweet) was not detected in the control group but was significantly elevated in the TP-containing rabbit meat, especially for 3 N-4, which corresponded to an increase of 88.1 %. The Cys levels decreased with aging past 5 years and higher TP additive amounts, and Cys was absent from the 10- and 15-year samples. This finding suggests a potential role in fat oxidation and the Maillard reaction. The threonine (Thr) level, which was initially at 8.55 in the control, dropped markedly in all samples following TP addition, particularly in 10 N-6, 15 N-4, and 15 N-6, where it decreased to 0.69, 0.98, and 0.98, respectively. Asp, which imparts an umami flavor to rabbit meat, had a low concentration in the original sample but rose by 31.6–39.0 % following TP addition, especially in 5 N-2, 5 N-4, 10 N-4, and 15 N-4.

Based on the above findings, incorporating different TP proportions and varying the aging durations can alter the amino acid profiles of rabbit meat. The heating process may cause certain amino acids to volatilize, such that they become volatile compounds and precursors to HAAs, which participate in addition or conversion reactions (Meurillon & Engel, 2016). Moreover, active constituents in TP, such as phenols and ketones, may neutralize free radicals during the frying of rabbit meat (Rafiq et al., 2018). This action prevents amino acids from engaging in high-temperature reactions with reducing sugars, creatine, and other meat components, thereby averting thermal decomposition and the subsequent formation of noxious substances (Jägerstad et al., 1991). Additionally, the interplay between the synergistic and antagonistic effects of amino acids contributes to these fluctuations.

3.3. Analysis of heterocyclic aromatic amines (HAAs)

3.3.1. Analysis of HAA types

Carcinogenic HAAs are produced in protein-rich food ingredients during frying (Wang et al., 2021). As shown in Fig. 4, the HAA

Table 3
Amino acid concentration in fried rabbit (mg/ 100 g).

Amino acids	Flavor characteristics	CG	3 N-2	3 N-4	3 N-6	5 N-2	5 N-4	5 N-6	10 N-2	10 N-4	10 N-6	15 N-2	15 N-4	15 N-6
Histidine (His)	Bitter	0.43 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Serine (Ser)	Sweet	22.94 ± 0.64	14.54 ± 0.90	14.92 ± 0.90	21.20 ± 0.17	19.35 ± 0.31	19.35 ± 0.33	13.15 ± 0.10	17.57 ± 0.94	21.68 ± 0.87	12.40 ± 0.89	14.48 ± 0.01	11.51 ± 0.58	9.24 ± 0.14
Arginine (Arg)	Sweet	245.18 ± 1.55	168.14 ± 0.75	179.27 ± 1.52	186.86 ± 1.68	231.02 ± 0.51	231.02 ± 0.06	213.07 ± 0.57	209.13 ± 0.47	209.18 ± 1.83	188.31 ± 0.23	198.64 ± 0.08	177.57 ± 1.06	167.24 ± 0.21
Glycine (Gly)	Sweet	73.65 ± 1.92	53.14 ± 0.17	53.44 ± 1.74	54.27 ± 0.19	69.84 ± 0.87	69.84 ± 0.90	57.69 ± 0.46	66.82 ± 1.12	66.44 ± 0.81	62.14 ± 0.39	62.77 ± 0.22	54.67 ± 0.15	53.38 ± 0.36
Aspartic acid (Asp)	Umami	2.46 ± 0.04	4.92 ± 0.03	5.86 ± 0.08	9.43 ± 0.13	12.17 ± 0.08	12.17 ± 0.07	5.73 ± 0.28	9.09 ± 0.32	10.24 ± 0.49	2.31 ± 0.26	8.49 ± 0.91	11.58 ± 0.12	8.69 ± 0.65
Glutamic acid (Glu)	Umami	46.17 ± 0.43	25.39 ± 0.70	30.57 ± 0.66	37.28 ± 0.30	43.04 ± 0.81	43.04 ± 0.99	33.02 ± 0.20	39.52 ± 0.44	50.25 ± 0.84	28.87 ± 0.24	37.27 ± 0.06	30.39 ± 0.54	26.86 ± 0.62
Threonine (Thr)	Sweet	8.55 ± 0.16	2.50 ± 0.07	2.85 ± 0.04	6.10 ± 0.01	5.80 ± 0.03	5.80 ± 0.02	2.94 ± 0.03	5.15 ± 0.06	6.55 ± 0.03	0.69 ± 0.02	3.23 ± 0.02	0.98 ± 0.07	0.98 ± 0.07
Alanine (Ala)	Sweet	71.21 ± 1.75	53.89 ± 0.37	55.66 ± 0.98	64.88 ± 1.72	68.63 ± 0.19	68.63 ± 0.81	55.68 ± 0.69	64.41 ± 0.83	73.89 ± 0.62	56.49 ± 0.81	63.45 ± 0.52	51.98 ± 0.36	46.39 ± 0.48
Proline (Pro)	Sweet	9.08 ± 0.08	15.77 ± 0.08	21.28 ± 0.40	24.43 ± 0.09	19.92 ± 0.70	18.92 ± 0.11	11.24 ± 0.04	25.07 ± 0.09	29.32 ± 0.17	18.56 ± 0.66	5.34 ± 0.22	3.28 ± 0.06	2.11 ± 0.07
Cystine (Cys)	Sweet	0.00 ± 0.00	0.63 ± 0.09	4.37 ± 0.14	1.98 ± 0.02	1.37 ± 0.08	1.27 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lysine (Lys)	Bitter	14.88 ± 0.45	4.72 ± 0.31	9.39 ± 0.46	14.51 ± 0.11	14.98 ± 0.12	14.98 ± 0.69	9.37 ± 0.60	13.81 ± 0.81	20.47 ± 0.64	8.19 ± 0.58	10.69 ± 0.89	7.16 ± 0.19	5.87 ± 0.17
Tyrosine (Tyr)	Bitter	1.36 ± 0.03	0.80 ± 0.03	1.54 ± 0.06	3.06 ± 0.04	0.84 ± 0.02	0.84 ± 0.06	0.00 ± 0.00	0.61 ± 0.04	1.62 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Methionine (Met)	Bitter	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.06	0.50 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.62 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Valine (Val)	Bitter	5.65 ± 0.09	1.10 ± 0.08	2.34 ± 0.07	4.87 ± 0.04	6.23 ± 0.18	6.23 ± 0.08	2.86 ± 0.03	4.91 ± 0.03	6.62 ± 0.09	0.67 ± 0.09	2.02 ± 0.01	2.70 ± 0.12	1.41 ± 0.02
Isoleucine (Ile)	Bitter	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Leucine (Leu)	Bitter	8.70 ± 0.60	3.84 ± 0.04	4.01 ± 0.03	8.41 ± 0.01	7.94 ± 0.02	7.94 ± 0.07	0.00 ± 0.00	3.36 ± 0.02	9.71 ± 0.20	2.37 ± 0.08	5.57 ± 0.09	2.05 ± 0.05	2.77 ± 0.00
Phenylalanine (Phe)	Bitter	0.14 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.68 ± 1.18	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total essential amino acids (EAA)		39.14	12.96	20.13	36.95	36.29	34.29	15.17	27.84	45.59	11.92	21.51	12.89	11.03
Total Free Amino Acid Content		510.25	349.38	385.49	437.27	501.63	460.57	404.76	459.48	507.73	381.00	411.96	353.86	324.94

±: means standard deviation (SD); CG: Control group; 3 N-2: 2 % aged three year; 3 N-4: 4 % aged three year; 3 N-6: 6 % aged three year; 5 N-2: 2 % aged five year; 5 N-4: 4 % aged five year; 5 N-6: 6 % aged five year; 10 N-2: 2 % aged ten year; 10 N-4: 4 % aged ten year; 10 N-6: 6 % aged ten year; 15 N-2: 2 % aged fifteen year; 15 N-4: 4 % aged fifteen year; 15 N-6: 6 % aged fifteen year.

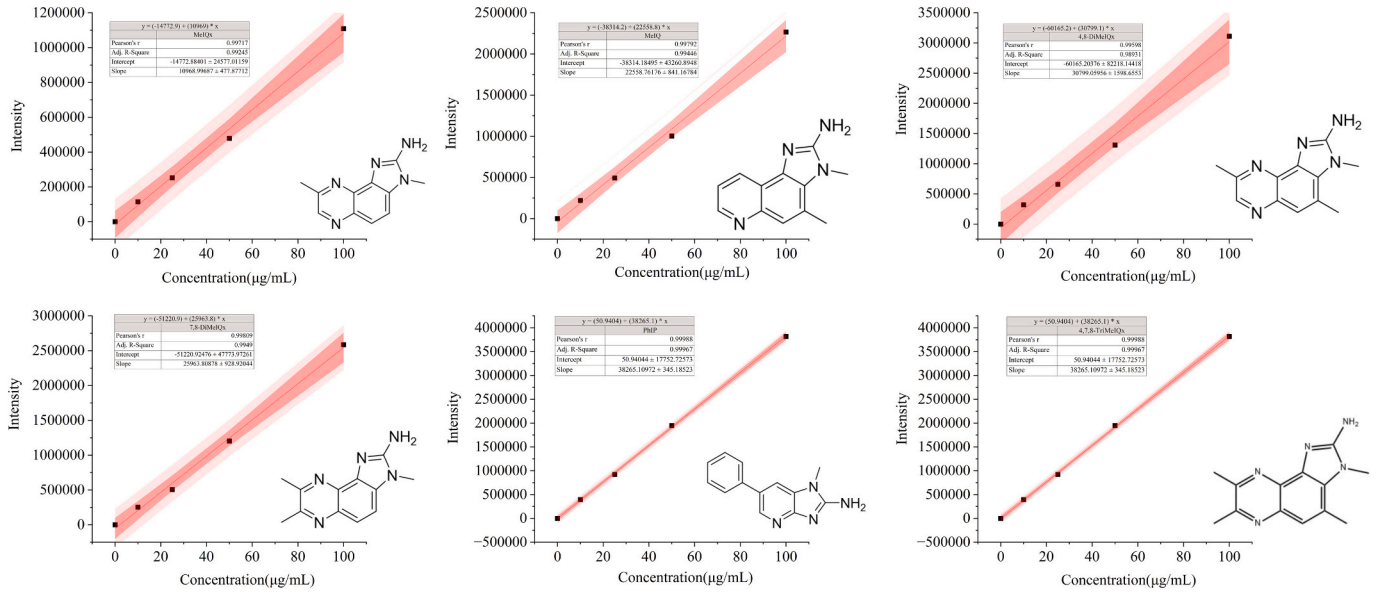


Fig. 4. Heterocyclic aromatic amine (HAA) calibration curve.

calibration curves showed good linearity in the range of 10–100 μg/mL with linear regression coefficients $R^2 > 0.98$. The limits of detection and quantification were in the ranges of 0.006–0.085 and 0.020–0.283 μg/mL, respectively. Table 4 reports the HAA concentrations in the DFRM; all five HAAs were detected for samples fried at 240 °C. The HAA content order (ascending) was 7,8-DiMeIQx, 4,8-DiMeIQx, MeIQ, MeIQx, and PhIP, where the content values ranged from 82.81 to 35.27 μg/kg; the total HAA content was 304 μg/kg.

In a comparative analysis of heat-generated HAAs in various animal-based ingredients, MeIQx and PhIP were absent from roast pork (Zhang, Wang, Chu, et al., 2023), whereas 7,8-DiMeIQx and 4,8-DiMeIQx were absent from roast fish (Zhang, Wang, and Zhou, 2023). Similarly, roast beef was found to lack 4,8-DiMeIQx and PhIP (Gumus and Kizil, 2022b), whereas another study reported the absence of MeIQx and 7,8-DiMeIQx from this ingredient (Zeng et al., 2014). As noted previously, the IARC classifies PhIP as a Class-2B carcinogen; further, PhIP is known to be one of the predominant HAAs present in cooked meats (Kızıl et al., 2011). Phenylacetaldehyde undergoes the Maillard reaction to produce phenylalanine, which subsequently reacts with creatinine to form PhIP (Kızıl et al., 2011). As detailed in Table 3, the absence of phenylalanine suggests a more efficient amino acid reaction, with additional intermediates for PhIP synthesis likely being provided. In the control samples, 7,8-DiMeIQx and 4,8-DiMeIQx were the most abundant HAAs, resulting mainly from pyrazine radical-compound intermediates generated by the Maillard reaction, which further reacted with acetaldehyde and creatinine (Gibis, 2016).

These results indicate a heightened propensity for carcinogen formation in DFRM. Thus, HAA mitigation during frying poses a substantial challenge.

3.3.2. *Analyzing TP suppression of HAAs in deep-fried rabbit meat (DFRM)*

Fig. 5 shows the effect of TP on HAA formation in DFRM. Overall, the TP significantly inhibited the HAA content and variety in the DFRM, with 5 N-4, 5 N-6, and 15 N-2 exhibiting inhibition rates of more than 94 %.

Fig. 5(a) reveals that, for 3-year TP at 2 %, 4 %, and 6 % concentrations, HAA Types 5, 3, and 3 formed, with total inhibition rates of 54 %, 82 %, and 78 %, respectively. At 4 % and 6 % concentrations, the MeIQ and MeIQx inhibition rates were 100 %. However, the inhibition was weaker for compounds with more methyl substitutions, such as 7,8-DiMeIQx, 4,8-DiMeIQx, and the phenyl ring-type PhIP. Except for 7,8-DiMeIQx, the HAA inhibition rates increased with TP additive content. Fig. 5(b) shows that, for 5-year TP at 2 %, 4 %, and 6 % concentrations, HAA Types 2, 1, and 1, respectively, formed. This outcome indicates significant inhibition of both the HAA variety and content. With 2 % addition, 100 % inhibition rates were obtained for MeIQ, MeIQx, and

Table 4
Concentration of heterocyclic aromatic amines in cooked rabbit meat (μg/kg) (n = 3).

Types	MeIQ	MeIQx	4,8-DiMeIQx	7,8-DiMeIQx	PhIP	Total	Inhibition%
CG	61.04 ± 20.85	58.72 ± 20.29	66.43 ± 24.56	82.81 ± 25.16	35.27 ± 2.67	305.27	–
3 N-2	43.09 ± 29.60	28.02 ± 19.08	23.73 ± 16.85	21.96 ± 15.51	22.53 ± 5.30	139.33	54.16 %
3 N-4	ND	ND	14.40 ± 24.93	20.07 ± 14.76	21.45 ± 13.27	54.01	81.61 %
3 N-6	ND	ND	12.49 ± 2.63	37.88 ± 12.97	16.92 ± 5.25	67.29	77.87 %
5 N-2	ND	ND	ND	57.95 ± 7.82	3.06 ± 1.30	61.01	79.94 %
5 N-4	ND	ND	ND	15.55 ± 6.93	ND	15.55	94.88 %
5 N-6	ND	ND	ND	17.81 ± 3.84	ND	17.81	94.14 %
10 N-2	ND	ND	ND	37.87 ± 12.97	ND	37.87	87.55 %
10 N-4	ND	ND	ND	53.43 ± 6.77	ND	53.43	82.44 %
10 N-6	ND	ND	ND	31.10 ± 6.93	ND	31.10	89.76 %
15 N-2	ND	ND	ND	17.81 ± 3.84	ND	17.81	94.14 %
15 N-4	ND	ND	ND	37.88 ± 16.92	ND	37.88	87.55 %
15 N-6	ND	ND	ND	37.88 ± 12.98	ND	37.88	87.55 %

Data included in this study are represented as the mean value ± standard error, based on three separate measurements. ND means not detected; CG: Control group; 3 N-2: 2 % aged three year; 3 N-4: 4 % aged three year; 3 N-6: 6 % aged three year; 5 N-2: 2 % aged five year; 5 N-4: 4 % aged five year; 5 N-6: 6 % aged five year; 10 N-2: 2 % aged ten year; 10 N-4: 4 % aged ten year; 10 N-6: 6 % aged ten year; 15 N-2: 2 % aged fifteen year; 15 N-4: 4 % aged fifteen year; 15 N-6: 6 % aged fifteen year.

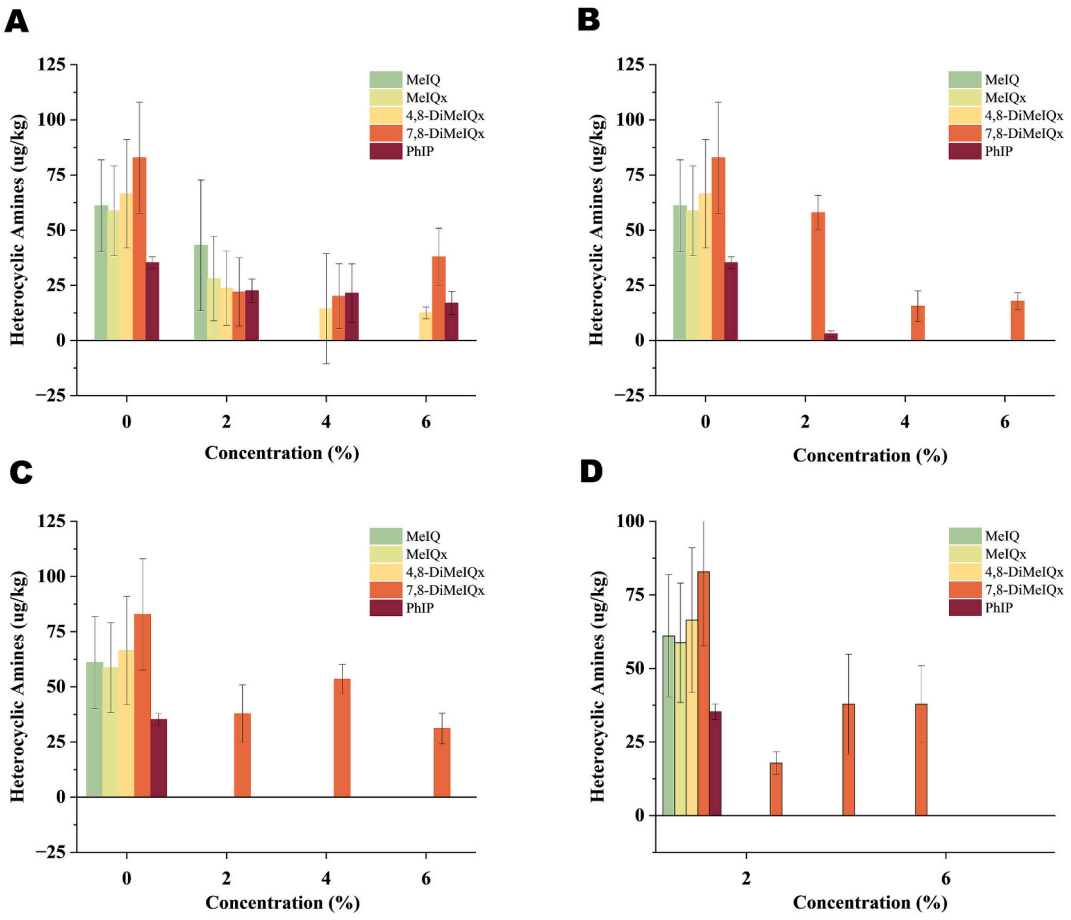


Fig. 5. HAA inhibition by TP aged 3, 5, 10, and 15 years at various additive amounts. HAA inhibition by **(a)** 3-, **(b)** 5-, **(c)** 10-, and **(d)** 15-year TP at 0, 2, 4, and 6 % additive amounts.

4,8-DiMeIQ, along with substantial inhibition of the phenyl ring-type PhIP, which reduced from 35.27 to 3.06 μg/kg. Increasing the TP concentration to 4 % and 6 % completely inhibited the MeIQ, MeIQx, 4,8-DiMeIQ, and PhIP growth, with the 4 % additive content yielding the highest inhibition rate of 94.88 % among the 12 samples tested. Fig. 5(c) shows the inhibitory effect of the 10-year TP on the HAAs. Compared to the 3- and 5-year samples, 10 and 15 years of aging increased the HAA inhibition to 80 %, with 2 % addition of 10-year TP yielding 100 % inhibition of MeIQ, MeIQx, 4,8-DiMeIQ, and PhIP.

Fig. 5(d) illustrates the inhibitory effect of 15-year TP on the HAAs. Consistent with the trends observed for the 10-year TP, 2 % TP addition was sufficient to completely inhibit the growth of MeIQ, MeIQx, 4,8-DiMeIQ, and PhIP. Additionally, the 7,8-DiMeIQx inhibition rate increased to 78 %. Analysis of the amino acids in the DFRM following TP addition revealed a significant increase in the total amino acid consumption. The amino acid content in the control group was 510.39 mg/100 g, whereas for the rabbit meat with 6 % 15-year TP, the HAA content decreased to 324.94 mg/100 g and the total amino acid consumption increased by 36 %, suggesting that more amino acids were involved in complicated chemical reactions. Notably, the Lys, histidine (His), Ser, Gly, and Arg consumption increased significantly. This outcome is consistent with the findings of Li et al., who reported accelerated consumption of Lys, Ser, and Gly during fruit extract-induced inhibition of HAAs in grilled yellow croaker (Li et al., 2022). MeIQx and 4,8-DiMeIQx formation is related to the presence of Lys, Ser, Ala, Gly, and Thr, whereas that of PhIP is related to the presence of isoleucine, phenylalanine, and Thr (Dong et al., 2020; Zöchling & Murkovic, 2002). However, it has also been reported that Maillard reaction products, such as Lys and Gly, have an inhibitory effect on HAAs (Linghu et al., 2017). According to this inhibitory process, in this study, Maillard intermediates such as phenylacetaldehyde, pyridine, or pyrazine radicals may have been inhibited by substances in the aged TP. Alternatively, TP addition may have altered the amino acid involvement in the reaction, as previously reported for reduced participation of sugars, creatine, and creatinine in the Maillard reaction (Li, Lin, et al., 2022). Thus, the HAA formation was reduced.

HAAs have been identified as mutagens using bacterial assays. MeIQ, MeIQx, PhIP, and 4,8-DiMeIQx are classified as IQ-type polar HAAs, and are frequently detected at cooking temperatures below 300 °C (Barzegar et al., 2018). Further, MeIQ, MeIQx, and PhIP are categorized as possible human carcinogens (Class 2B, as noted previously) and pose significant health risks. MeIQ and MeIQx are the most commonly occurring HAAs in beef products cooked using different methods (Bulan & Oz, 2021). In roast beef, lamb chops, and venison, 4,8-DiMeIQx is the most prevalent HAA (Szterk, 2015). PhIP, the most abundant HAA in the human diet, can modulate gene expression through interaction with ER-α (a genomic pathway) and activate cell signaling pathways (non-genomic) such as ERK phosphorylation, causing DNA damage and cell mutations. Recent research has focused on inhibiting MeIQ, MeIQx, PhIP, and 4,8-DiMeIQx growth through the addition of exogenous substances. Cheng et al. (2021) reported that the addition of sugarcane (*Saccharum officinarum L.*) molasses extract to deep-fried chicken wings yielded MeIQ, MeIQx, 4,8-DiMeIQx, and PhIP inhibition rates of 39.8 %, 25.2 %, 45.3 %, and 62.2 %, respectively. Xue et al. (2020) discovered that His inhibited MeIQx, PhIP, and 4,8-DiMeIQx in beef patties by 7.48 %, 47.69 %, and 18.18 %, respectively, with the MeIQ inhibition rate increasing by 59.26 %. Oz and Cakmak (2016) found that the addition of conjugated linoleic acid to beef meatballs during frying inhibited the total HAA

content by up to 60 % at the highest addition level. Similarly, Zhang et al. (2020) found that the use of chitosan in beef patties oven-roasted at 250 °C yielded a 45 % inhibition rate for PhIP, with no significant decrease in the MeIQx and 4,8-DiMeIQx content.

Comparative studies have indicated that exogenous additives can inhibit HAA formation; however, the achieved inhibition rates fall short of consumer expectations. Here, TP incorporation has been shown to completely inhibit the synthesis of mutagenic compounds such as MeIQ, MeIQx, PhIP, and 4,8-DiMeIQx. Therefore, this study presents a natural and safe method for mitigating the carcinogenic risks associated with fried meat consumption.

3.4. Correlation between amino acids, VOCs, and HAA formation

Fig. 6(a) is a heat map of the correlation between the amino acids and heterocyclic amines in rabbit meat. His was significantly positively correlated with MeIQ, MeIQx, 4,8-DiMeIQx, and 7,8-DiMeIQx ($P < 0.001$), and positively correlated with PhIP ($P < 0.01$). This suggests that His can effectively suppress the five IQ-type HAAs. Note that Xue et al. (2021) found that the addition of 1 % His effectively inhibited quinoline-type heterocyclic amines in grilled beef patties. The Arg levels were positively correlated with the PhIP levels ($P < 0.001$). Further, leucine (Leu), Thr, and Gly were positively correlated with PhIP ($P < 0.01$), as were Ser, Glu, and Ala ($P < 0.05$). Asp, Pro, and methionine (Met) were negatively correlated with MeIQ, MeIQx, 4,8-DiMeIQx, and PhIP ($P < 0.001$). PhIP formation involves the following five steps: Step 1: creatinine reacts with active carbonyl compounds; Step 2: a thermal reaction occurs between the creatinine and carbonyl compounds, causing the formation of an intermediate known as aldol; Step 3: the aldol intermediate loses a water molecule in the dehydration reaction, forming a new compound; Step 4: the resulting compound undergoes a ring-closing reaction in the presence of ammonia and formaldehyde, causing the formation of a pyridine ring; Step 5: completion of the ring-closing reaction causes the formation of aminomethylimidazole and aromatic hydrocarbon PhIP (Oz et al., 2023; Zamora et al., 2014). Previous research has indicated that His, Leu, Try, and Lys can inhibit the formation of heterocyclic amines in high-temperature meat products through various mechanisms, such as the scavenging of benzaldehyde and free radicals, thereby forming benzaldehyde– and heterocyclic amine–amino acid adducts with benzaldehyde and heterocyclic amines, respectively, and exerting competitive inhibitory effects (Linghu et al., 2017).

Fig. 6(b) is a heatmap of the correlation between the VOCs and heterocyclic amines. Perilla aldehyde, nonanal, and (−)-cis-carveol were extremely significantly positively correlated with MeIQ, MeIQx, and 4,8-DiMeIQx ($P < 0.001$), and significantly positively correlated with PhIP ($P < 0.01$). Proteins can undergo the Strecker degradation reaction at high temperatures, causing aldehyde formation. Aldehydes are important substrates for the formation of certain hazardous factors (heterocyclic amines and polycyclic aromatic hydrocarbons). In cooked meat products, amino-imidazo-azaarenes mostly cyclize to form creatinine at high temperatures of approximately 200 °C. The pyridine and pyrazine generated during the Maillard reaction react with creatinine and aldehyde compounds to form heterocyclic amines with different structures (Aaslyng et al., 2013). Here, sabinene was significantly negatively correlated with MeIQ, MeIQx, 4,8-DiMeIQx, and PhIP. This indicates that sabinene contributes to the inhibition of IQ-type HAAs, a behavior that may be related to its strong free-radical scavenging ability (Ruberto & Baratta, 2000). Limonene is automatically oxidized under high-temperature conditions to generate a series of oxidized monoterpenes, such as limonene oxide, (−)-cis-carveol, and limonene dihydroperoxide (Duetz et al., 2003). Correlation analysis suggests that the generation of (−)-cis-carveol is related to an increase in heterocyclic amines. Previous studies have reported that, at high temperatures, alcohols may react to generate aldehydes or ketones, thereby accelerating HAA formation (Yang et al., 2019).

4. Conclusion

To the best of our knowledge, this is the first study to examine the influence of TP on HAA formation in DFRM. This study investigated the impact of and correlation between the TP aging year and additive quantity and the flavor, HAAs, and precursor substances of DFRM. The majority of VOCs were detected in the 3-year TP, with the compound

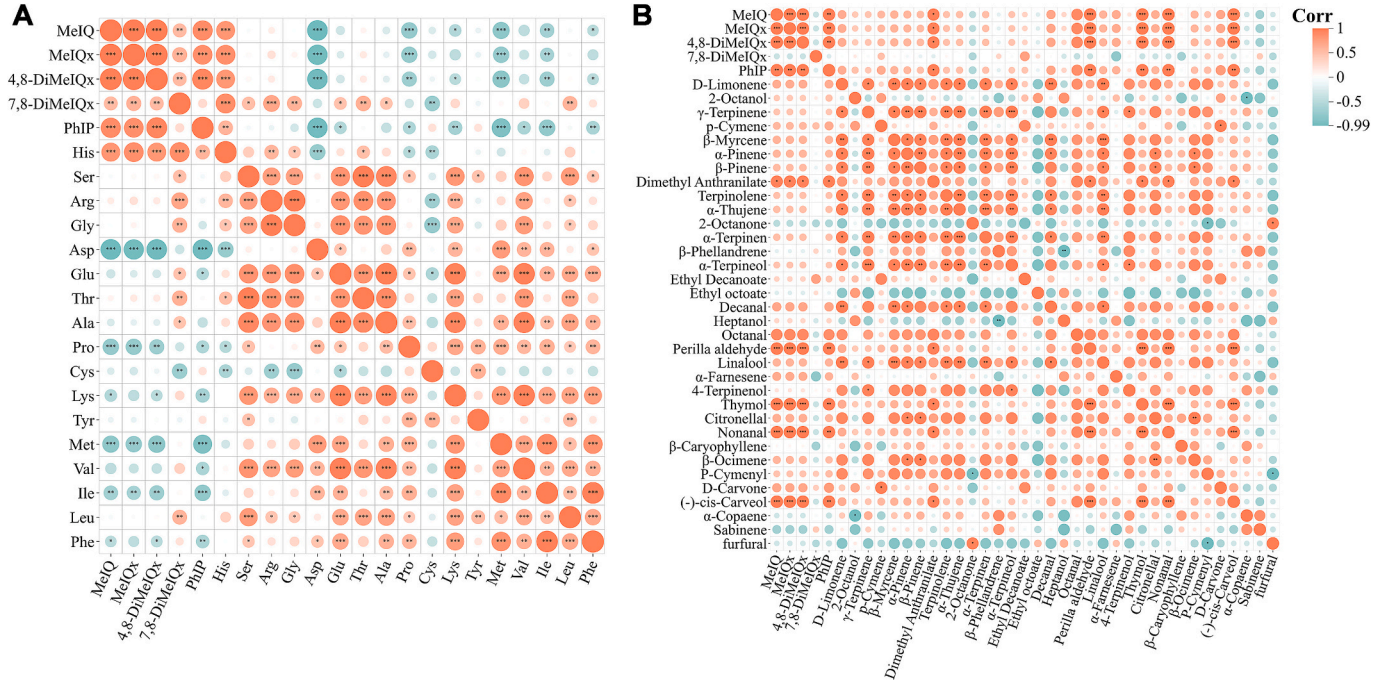


Fig. 6. Correlation analysis of amino acids and VOCs with HAA formation. Correlation between **(a)** amino acids and HAA formation and **(b)** VOCs in TP and HAA formation.

concentrations in the TP decreasing as aging progressed. This decline was particularly noticeable in the 15-year samples, with D-limonene and γ-terpinene exhibiting significant downward trends. Interestingly, allyl caprylate, thymol, and (−)-cis-carveol were identified as unique compounds in the 3-year TP, whereas furfural was unique to the 15-year TP. Monitoring these distinct VOCs can help mitigate consumer fraud. Assessment of the HAA concentration in the DFRM revealed that the presence of TP markedly diminished the IQ-type HAA variety and concentration. With the exception of 5 N-2, TP aged for 5, 10, and 15 years inhibited the formation of MeIQ, MeIQx, 4,8-DiMeIQx, and PhIP by 100 %. The HAA inhibitory capacity did not exhibit a clear TP dose dependence. Correlation analysis revealed a strong positive correlation between the His, aldehydes, and alcohols, and the IQ-type HAAs ($P \leq 0.001$). The insights gleaned from this study are expected to provide valuable guidance for TP application as a potential HAA inhibitor in deep-fried meat products.

CRediT authorship contribution statement

Chunyuan Ping: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Xiangdong Zhao:** Methodology, Data curation. **Congcong He:** Validation, Supervision. **Yingying Zheng:** Validation. **Haibao Zhang:** Supervision, Methodology, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101902.

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