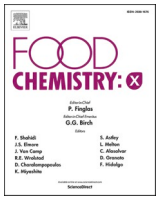
Food Chemistry: X 14 (2022) 100315

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effect of acrolein, a lipid oxidation product, on the formation of the heterocyclic aromatic amine 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP) in model systems and roasted tilapia fish patties

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ARTICLE INFO

Keywords:
Acrolein
PhIP
Intermediates
Adduct
Roasted tilapia fish

ABSTRACT

The effect of acrolein on the formation of the 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) was investigated in a chemical model. Acrolein was found to increase PhIP formation at each tested addition level. 0–0.2 mmol of acrolein increased PhIP formation dose-dependently, while high levels of acrolein (>0.2 mmol) did not further increase PhIP formation. Mechanistic study showed that acrolein addition decreased the residue of phenylalanine and creatinine, but increased the content of some key intermediates. Further analysis indicated that acrolein can react with phenylalanine, creatinine, and PhIP to form adducts. These results suggested that acrolein was able to contribute to PhIP formation as a consequence of its comprehensive ability to facilitate Strecker degradation of phenylalanine and react with phenylalanine, creatinine, and PhIP. In addition, oxidation of the tilapia fish increased the PhIP formation in the roasted fish patties, further supporting the potential contribution role of lipid oxidation products to the formation of PhIP.

Introduction

Heterocyclic aromatic amines (HAAs), mainly generated during thermal processing of protein-rich foods such as meat and fish, are well-known mutagens and rodent carcinogens (Alaejos & Afonso, 2011; Dong, Xian, Li, Bai, & Zeng, 2020; Yang, Ji, Wang, Fan, Zhao, & Wang, 2021; Fan et al., 2018). So far, more than 30 HAAs have been identified in various foods. Several epidemiological studies indicated that the frequent intake of thermally processed foodstuffs containing HAAs may lead to an increased risk of human cancers, such as colon, prostate, and mammary cancers (Alaejos & Afonso, 2011; Cao et al., 2020; Liu, Xia, Hu, Ni, Thakur, & Wei, 2020; Xu et al., 2021). The International Agency for Research on Cancer (IARC) has thus classified IQ as a probable (class 2A) human carcinogen and PhIP, MeIQx, and MeIQ as possible (class 2B) human carcinogens (IARC, 1993).

The pathways involved in the formation of HAAs have been the objective of numerous studies, so as to develop some targeted strategies to control their formation. PhIP has been mostly studied in this aspect, as it is generally the most abundant HAAs in normal thermally processed foods (Ni, McNaughton, LeMaster, Sinha, & Turesky, 2008). Although the complete formation mechanism is still not fully established, Strecker degradation (decarboxylation and deamination reaction of amino acid) of phenylalanine with phenylalanine and mono-sugars as the initial reactants is believed to be the first step for PhIP formation. The generated degradation product, phenylacetaldehyde then condenses with creatinine to form PhIP (Jägerstad, Skog, Arvidsson, & Solyakov, 1998; Zamora & Hidalgo, 2015; Zhao, Yang, Zhang, Zhou, Fan, & Wang, 2021; Zöchling and Murkovic, 2002). It should be noted that phenylacetaldehyde can be produced by thermal decomposition of phenylalanine directly, different carbonyl compounds produced by sugar decomposition might facilitate this reaction (Estévez, Ventanas, & Heinonen, 2011; Zamora, Delgado, & Hidalgo, 2012; Zamora & Hidalgo, 2015).

Unsaturated lipids in foods are susceptible to rapid oxidative deterioration upon thermal treatment, resulting in the production of a wide variety of oxidation products, including lipid hydroperoxides and

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https://doi.org/10.1016/j.fochx.2022.100315
Received 12 February 2022; Received in revised form 14 April 2022; Accepted 20 April 2022
Available online 23 April 2022
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reactive carbonyl species (RCS) (Liu, Zhu, Liu, Fan, Wang, & Zhao, 2021; Zamora & Hidalgo, 2016; Zhang et al., 2021; Zhao, Zhang, Zhang, Zhou, Fan, & Wang, 2022). Slight to medium oxidized soybean oil has been found to contribute to PhIP formation, while highly oxidized oils decreased the formation of PhIP in a chemical model (Zamora, Alcón, & Hidalgo, 2012). This was thought to due to the fact that, like carbohydrates, the lipid oxidation products formed at different stages of lipid peroxidation namely primary, secondary, and tertiary products of lipid oxidation could help convert phenylalanine into phenylacetaldehyde by Strecker degradation, thus contributing to the formation of PhIP, while excessive lipid oxidation produced lipid polymers which do not contribute to PhIP formation (Zamora, Alcón, & Hidalgo, 2012). However, it should be noted that, theoretically, except for participating in Strecker degradation, the carbonyl group of the lipid oxidation products could also react with the amino group of amino acid, creatinine, PhIP, and some intermediates of PhIP (e.g. aldol condensation product), thus we hypothesize that the effect of lipid oxidation products on PhIP formation should be a consequence of the comprehensive ability of the carbonyl compound to facilitate Strecker degradation of phenylalanine and reacting with the nucleophilic groups of PhIP and some of its precursors and intermediates.

Acrolein is a reactive α,β-unsaturated aldehyde commonly generated during food thermal processing as a consequence of lipid oxidation (Zhu et al., 2009). This study aimed to examine the effects of acrolein on PhIP formation in a PhIP-producing chemical model containing the obligatory precursors, phenylalanine and creatinine. To clarify the underlying mechanism, the effects of acrolein on PhIP precursors and some key intermediates were further evaluated in the chemical model. The reaction between acrolein and phenylalanine, glucose, creatinine, and PhIP were also analyzed, respectively. To further evaluate the effect of lipid oxidation on the formation of PhIP, the PhIP formed in the tilapi fish oxidized for different times were measured after roasting.

Materials and methods

Reagents and chemicals

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) was purchased from Toronto Research Chemicals (North York, Ontario, Canada). Acrolein (ACR) and phenylacetaldehyde were obtained from Aladdin Biochemical Technology Co., Ltd. (Shanghai, P.R. China). Creatinine and phenylalanine were purchased from Macklin Company (Shanghai, P.R. China). Methanol of HPLC grade was obtained from Merck & Co., Inc. (New Jersey, USA). Other solvents of analytical grade were obtained from Shanghai ANPEL Scientific Instrument Co., Ltd. (Shanghai, P.R. China). 10 mL Pyrex® glass tubes with a PTFE-lined screw-cap were obtained from Shanghai Leigu Instrument Co., Ltd. (Shanghai, P.R. China).

Effects of acrolein on the formation of PhIP in a PhIP-producing chemical model

A PhIP-producing chemical model containing the obligatory precursors phenylalanine (40 mM) and creatinine (40 mM) in 5 mL of distilled water in screw cap-sealed reaction vials was applied to evaluate the effect of acrolein on PhIP formation (Han et al., 2017; Zamora, Alcón, & Hidalgo, 2012). Different amounts of acrolein (0, 0.02, 0.04, 0.08, 0.16, 0.2, and 0.4 mmol) were added to the model system, and heated at 130 °C in screw cap-sealed tubes for 2 h. After cooling down, 2 mL of reaction mixture was extracted with 8 mL of ethyl acetate by vortexing for 2 min and ultrasonication for 15 min. The two-phase mixtures were then centrifuged for 10 min at 4000 × *g*, and the clear ethyl acetate supernatant was collected in a round-bottom flask. The above extraction procedure was repeated for three times. The extraction solutions were combined and evaporated to a minimum volume using a rotary evaporator under vacuum at 40 °C. The resulting residue was dissolved in 1 mL of methanol, sonicated, and filtered before PhIP determination by UPLC-MS.

UPLC-MS analysis of PhIP was performed on a Waters Acquity UPLC system coupled to a triple quadrupole mass spectrometer equipped with ESI. Samples were separated on a Waters Atlantis dC18 column (4.6 × 150 mm, i.d., 3.0 μm). Mobile phase was comprised of methanol (A) and 0.1% formic acid in water (B). The gradient with a flow rate of 0.35 mL/min was as follows: 0 min, 10% A; 1.0 min, 10% A; 4.0 min, 100% A; 5.0 min, 100% A; 5.1 min, 10% A; 7.0 min, 10% A. The ESI-MS spectrometer conditions were as follows: positive electrospray ionization mode; electrospray voltage, 3.5 kV; capillary temperature, 350 °C; collision energy, 20 eV; mass scan range, 50–1800 *m/z*. Mass spectrometric detection was performed with multiple reaction monitoring (MRM) mode. m/z = 225.0 → 140.0 was used as qualifying ions and m/z = 225.0 → 210.0 as quantifying ions to detect PhIP. PhIP was identified by comparing the retention time and MS spectrum with commercial standard. Quantitative determination of PhIP was performed using a calibration curve (y = 1485.3x + 1807.5, R^2 = 0.9996) plotted at eight calibration levels (0, 1, 5, 10, 20, 50, 100, 200 ng/mL). The limit of detection (LOD) and the limit of quantification (LOQ) values of PhIP were detected to be 0.05 ng/mL and 0.18 ng/mL according to signal-to-noise ratios of 3 and 10, respectively. The recovery and precision of the proposed method were determined by spiking blank samples with a certain amount of PhIP standard. Three replicates were carried out, and standard deviation was calculated as precision. The average recovery and precision for PhIP were 85.56% and 2.73%, respectively, calculated using the following formula: Recovery (%) = [(amount calculated from standard curve) – (original amount)] / spiked amount × 100%.

Effects of acrolein on the content of precursors and intermediates of PhIP in a PhIP-producing chemical model

HPLC analysis of phenylalanine and creatinine

The reaction mixture from section 2.2 was diluted 4-fold with Milli-Q water and filtered through a 0.22 μm membrane before phenylalanine and creatinine determination by a HPLC system (e2695, Waters) equipped with a PDA detector (2998, Waters) (Wijemanne, Soysa, Wijesundara, & Perera, 2018). Separation was performed on a YMC-Pack ODS C18 column (250 mm × 4.6 mm, 5 μm) The mobile phase was composed of methanol (A) and 0.1% formic acid in water (B) with a flow rate of 1 mL/min in the following gradients: 0–15 min, 90% B. The PDA was set at 256 and 235 nm for phenylalanine and creatinine analysis, respectively. Phenylalanine and creatinine were identified by comparing the retention time with commercial standards. Quantitative determination of phenylalanine and creatinine were performed using calibration curves plotted for each compound at six calibration levels (0, 200, 400, 600, 800, and 1000 μg/mL). Calibration curves were as follows: y = 16712x – 16356, R^2 = 0.9997 (phenylalanine); y = 17173x – 699840, R^2 = 0.9996 (creatinine). LOD of phenylalanine and creatinine was 0.25 and 0.45 μg/mL, respectively. LOQ of phenylalanine and creatinine was 0.80 and 1.65 μg/mL, respectively. The average recovery of phenylalanine and creatinine were 88.4% and 82.5%, respectively. The average precision of phenylalanine and creatinine were 3.2% and 3.6%, respectively.

UPLC-MS analysis of phenylacetaldehyde

Phenylacetaldehyde content in the PhIP-producing chemical model was measured according to previous studies (Yang, Ji, Wang, Fan, Zhao, & Wang, 2021; Zhao, Yang, Zhang, Zhou, Fan, & Wang, 2021). Briefly, 0.5 mL of the reaction mixture from section 2.2 was mixed with 0.5 mL of *o*-phenylenediamine (OPD, 5 mM) thoroughly and kept on an oscillator at room temperature for 24 h in the dark for phenylacetaldehyde derivatization. The mixture was then filtered with 0.22 μm membrane before injected into the UPLC-MS system. UPLC-MS analysis was performed using the same condition as applied for PhIP analysis. m/z 166.0 and 120.0 were selected for the quantification of the related

derivatization product. $m/z = 209.0 \rightarrow 166.0$ was used as qualifying ions and $m/z = 209.0 \rightarrow 120.0$ as quantifying ions to detect the derivatization product of phenylacetaldehyde. The LOD and LOQ values of phenylacetaldehyde were detected to be 0.07 ng/mL and 0.21 ng/mL, respectively. The average recovery and precision of phenylacetaldehyde were 84.7% and 3.7%, respectively.

UPLC-MS analysis of the aldol condensation product

An aldol condensation product is formed between creatinine and phenylacetaldehyde (Yu and Yu, 2016; Zöchling and Murkovic, 2002). To analyze the effect of acrolein on the aldol condensation product, the PhIP-producing chemical model and the extraction procedure were the same as described in section 2.2. The aldol condensation product was analyzed by UPLC-MS using the same conditions as applied for PhIP determination. $m/z = 216.1 \rightarrow 81.5$ was used as qualifying ions and $m/z = 216.1 \rightarrow 191.1$ was used as quantifying ions to detect the aldol condensation product (Yang, Ji, Wang, Fan, Zhao, & Wang, 2021; Yu and Yu, 2016). The relative amount of the aldol condensation product was calculated based on the proportion of peak area in the chromatograms.

Reaction between acrolein and phenylalanine or between acrolein and creatinine

Analysis of reaction products between acrolein and phenylalanine or between acrolein and creatinine in the acrolein-phenylalanine and acrolein-creatinine model system by a semi-preparative HPLC

Phenylalanine (0.2 mmol) or creatinine (0.2 mmol) was mixed with different amounts of acrolein (0, 0.02, 0.04, 0.08, 0.16, 0.2, 0.4 mmol) in 5 mL of distilled water in screw cap-sealed tubes. The mixture was heated at 130 °C for 2 h and further analyzed by semi-preparative HPLC system (Shimadzu) to identify reaction products (new peaks) formed in the model system (Wang, Tao, Zhu, Fan, Wang, & Zhao, 2021). Briefly, separation was performed on a SunFire Prep C18 OBD column (5 μm, 250 mm × 19 mm) eluted with water (solvent A) and acetonitrile (solvent B) with a flow rate of 5 mL/min. The gradient program was as follows: 0–5 min, 10–25% B; 5–10 min, 25–40% B; and 10–30 min, 40–50% B. The injection volume was 100 μL. The eluents were monitored at 256 nm and 235 nm, respectively.

Isolation of reaction products between acrolein and phenylalanine or between acrolein and creatinine in the acrolein-phenylalanine and acrolein-creatinine model system by semi-preparative HPLC

Phenylalanine (0.2 mmol) or creatinine (0.2 mmol) was mixed with acrolein (0.2 mmol) in 5 mL of distilled water, respectively. After heated at 130 °C for 2 h, the mixture were isolated and collected by semi-preparative HPLC on a Shimadzu HPLC system using the same conditions as described in section 2.4.1. Each fraction was collected and further analyzed on a MS/MS system.

MS analysis of reaction products

The reaction products were analyzed on an Agilent 1290 UPLC system equipped with a Q-TOF 6550 mass spectrometer (Agilent technologies, California, USA). Separation was carried out on a Waters BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm). The mobile phase was composed of 0.1% formic acid in water (solvent A) and methanol (solvent B). The gradient program (0.3 mL/min) was as follows: 0–14 min, 90% A. The MS conditions: positive ion mode; mass range, 50–1000 m/z; capillary temperature, 350 °C; capillary voltage, 4 kV; collision energy, 20 eV.

Analysis of the reaction product between acrolein and PhIP in the acrolein-PhIP model by UPLC-MS

PhIP (7 nmol) was mixed with different amounts of acrolein (0, 0.02, 0.04, 0.08, 0.16, 0.2, and 0.4 mmol) in 5 mL of distilled water. After heated at 130 °C for 2 h, the mixture was filtered through a 0.22 μm membrane for UPLC-MS analysis. UPLC-MS conditions were the same as described in section 2.2. m/z 281/263 and m/z 281/225 were selected for the quantification of reaction product between acrolein and PhIP (Vanhaecke et al., 2006; Zhang, 2018).

Oxidation of tilapia fish

Thiobarbituric acid reactive substance (TBARS) analysis

TBARS value is an index used as a marker of lipid oxidation. TBARS value of the minced tilapia fish preserved at 4 °C for 0–9 days in a refrigerator was measured according to previous studies (Soladoye et al., 2017; Zhao, Kong, Zhang, Hu, & Wang, 2019). Briefly, 2.0 g of tilapia fish were extracted with 25 mL of 5% trichloroacetic acid (TCA) solution for 5 min. The mixture was centrifuged at 4,000 × g for 15 min to obtain the supernatant (TBARS). Then, 5 mL of 0.02 M TBA was added to 5 mL of the supernatant. After heating at 100 °C for 60 min, total malondialdehyde (MDA) in samples was measured spectrophotometrically at 532 nm on a UV-2300 ultraviolet spectrophotometer (Shanghai Techcomp Ltd.). Quantitative determination of MDA was performed using a calibration curve ($y = 1.4915x - 0.0523$, $R^2 = 0.9998$) built by different concentrations of standard solutions (1,1,3,3-Tetraethoxy-propane, 0.01–0.25 μg/mL). The TBARS value was calculated using the following formula: TBARS value (mg MDA/kg meat) = (c × V)/m, where c represents the concentration of MDA in extract obtained from the standard curve (μg/mL), V represents the volume of TCA solution used for extraction (mL), and m represents the mass of the sample (g).

Analysis of aldehydes in minced tilapia fish by GC–MS

The volatile aldehydes in the minced tilapia fish were measured by GC–MS according to previous studies (Iglesias, Gallardo & Medina, 2010; Li, Fan, Zhao, & Wang, 2020; Xu et al., 2022). Briefly, 2.5 g of minced tilapia and 25 μL of internal standard 2,4,6-trimethylpyridine (1 μg/mL) were homogenized with 5 mL of 0.18 g/mL NaCl solution for 2 min. A solid-phase microextraction (SPME) fiber coated with PDMS/DVB (65 μm) adsorbed the volatile *o*-2,3,4,5,6-(Pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) was placed in the headspace of each vial containing 2.5 g of homogenized fish mixture and maintained for 30 min for aldehydes derivatization. The fiber was then desorbed at 250 °C for 5 min in the injection port of an Agilent 6890 gas chromatography (GC) system equipped with an Agilent 5973 Mass detector. Separation was carried out on an HP-5 MS 5% phenyl methyl siloxane column (30.0 m × 250 μm × 0.25 μm). The operating conditions were as follows: column temperature program, 30 °C (1 min), 30 to 120 °C at 10 °C min^{-1}, 120 to 250 °C at 15 °C min^{-1}, 250 °C (3 min); carrier gas, helium at 1.00 mL min^{-1}; injection temperature, 250 °C; injection mode, splitless. The MS conditions were as follows: ionization mode, electron ionization; ionization voltage, 70 eV; ion source temperature, 280 °C. Aldehydes were identified by comparing the retention time and MS spectrum with commercial standards. Aldehydes were quantified by means of calibration curves formed from known concentrations of mixtures of analyte standards with a constant level of the internal standard. These standards were spiked into fresh meat and subjected to the normal sample preparation procedure. Seven calibration levels were used (0, 1, 10, 20, 50, 100, and 250 ng/g meat). The concentration of internal standard in meat was 1 ng/g meat. Quantification was based on peak area ratios related to the internal standard.

UPLC-MS analysis of PhIP in roasted tilapia fish patties

The roasted tilapia fish patties were prepared according to our previous studies (Yang, Ji, Wang, Fan, Zhao, & Wang, 2021; Zhao, Yang, Zhang, Zhou, Fan, & Wang, 2021). Briefly, the tilapia fish patties (10 g) formed in a disk shape using a glass petri dish (4 × 0.4 cm) were roasted in an oven at 250 °C for 5 min on each side. The obtained tilapia fish patties were homogenized with 20 mL of 1 M NaOH to form a dense paste. Then, the paste was mixed with 20 mL of *n*-hexane to dissolve

lipids into *n*-hexane. The *n*-hexane phase was discarded to remove the lipids by centrifugation at 4000 × *g* for 8 min. After that, PhIP in the tilapia fish paste was extracted using the same protocols as described in the chemical models (section 2.2), except that 20 mL of ethyl acetate was used in each extraction step. UPLC-MS analysis was performed using the same conditions as described in section 2.2.

Statistical analysis

All data are presented as the mean ± standard deviation (SD) from triplicate analysis. The data were analyzed by analysis of variance (ANOVA) and Duncan test. Statistical analyses were performed using IBM SPSS 25.0 for Windows (SPSS Inc., Chicago, IL, USA), and values were considered to be statistically significant at $P < 0.05$.

Results and discussion

Effects of acrolein on the formation of PhIP in a PhIP-producing chemical model

The effects of different concentration (0 to 0.4 mmol) of acrolein on PhIP formation was evaluated in a PhIP-producing chemical model containing the obligatory precursors phenylalanine (0.2 mmol) and creatinine (0.2 mmol) in 5 mL of distilled water in screw cap-sealed reaction vials heated at 130 °C for 2 h. As shown in Fig. 1, each addition level of acrolein increased the formation of PhIP in the chemical model. Specifically, when addition level of acrolein was increased from 0 to 0.2 mmol, the PhIP formed in the chemical model was significantly increased with increased addition of acrolein (e.g. 0.02, 0.04, 0.08, 0.16, and 0.2 mmol of acrolein increased the formation of PhIP by 102%, 319%, 476%, 622%, and 579%, respectively); when the addition of acrolein was higher than 0.2 mmol, PhIP formed in the chemical model decreased compared with 0.2 mmol addition level but still significantly higher than the control group (e.g. 0.4 mmol of acrolein increased the formation of PhIP by 307%). Our results were in consistent with a previous study, where primary, secondary, and tertiary lipid oxidation products such as MeLOOH, MeLnOOH, 2-octenal, 2-pentenal, 4-oxo-2-nonenal, and 4-oxo-2-hexenal were found to significantly increase the formation of PhIP (Zamora, Alcón, & Hidalgo, 2012). However, only one concentration of lipid oxidation products was tested in the previous study and they did not study the concentration effect. These results suggest that a certain level of acrolein was able to contribute to PhIP formation, while the contribution effect was inhibited when high levels acrolein was present.

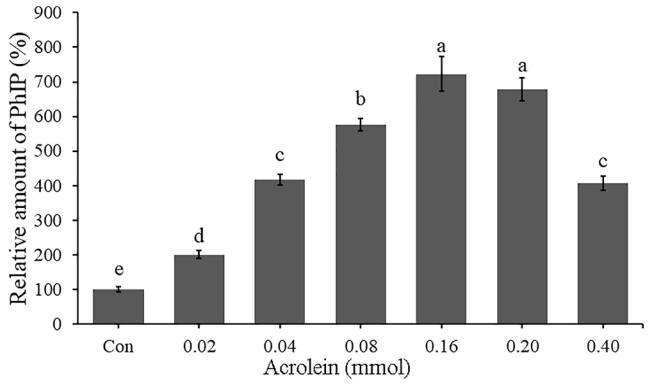

Fig. 1. Effects of different concentration of acrolein on the formation of PhIP in a PhIP-producing chemical model. 0.2 mmol phenylalanine and 0.2 mmol creatinine with the addition of 0, 0.02, 0.04, 0.08, 0.16, 0.2, and 0.4 mmol were mixed in 5 mL of distilled water in screw cap-sealed reaction vials and heated at 130 °C for 2 h. PhIP was determined as described in section 2.2. Values are mean ± standard deviation, n = 3. Different letters (a-e) within a column indicate significant differences among groups ($P < 0.05$).

Effects of acrolein on PhIP precursors and intermediates in a PhIP-producing chemical model

In an attempt to understand the increase of PhIP formation when acrolein was added to the model system, the effect of different concentrations of acrolein on the content of precursors (phenylalanine and creatinine) and the key intermediates (phenylacetaldehyde and aldol condensation product) of PhIP was evaluated in the PhIP-producing chemical model. As shown in Fig. 2, acrolein addition decreased the residue of phenylalanine (Fig. 2A) and creatinine (Fig. 2B) in a concentration dependent manner; the change tendency caused by acrolein on intermediates is similar to that on heterocyclic amines (the addition of acrolein increased the content of phenylacetaldehyde and aldol condensation product at 0 to 0.2 mmol but decreased the content of theses intermediates at the concentration more than 0.2 mmol) (Fig. 2C and 2D). These results indicated that acrolein at low addition levels (0–0.2 mmol) primarily facilitates the Strecker degradation of phenylalanine to produce more phenylacetaldehyde and the so generated phenylacetaldehyde condenses with more creatinine (lead to less creatinine residue) to form more aldol condensation product and subsequently more PhIP. When the addition level of acrolein was more than 0.2 mmol, more acrolein might react with phenylalanine, creatinine, and PhIP, thus leading to the decreased formation of PhIP compared with 0.2 mmol addition of acrolein, because the electrophilic carbonyl group of acrolein could theoretically react with the nucleophilic amino group of phenylalanine, creatinine, and PhIP.

Reaction between acrilein and phenylalanine, creatinine, and PhIP in the acrolein-phenylalanine, acrolein-creatinine, and acrolein-PhIP model

The above-described results suggested that the effect of acrolein on PhIP formation was a consequence of the comprehensive ability of acrolein to facilitate Strecker degradation of phenylalanine and reacting with phenylalanine, creatinine, and PhIP. To further confirm this hypothesis, we next studied the reaction between acrolein and phenylalanine, creatinine, and PhIP in the acrolein-phenylalanine, acrolein-creatinine, and acrolein-PhIP model, respectively. As shown in Fig. 3A, the addition of acrolein decreased the content of phenylalanine in a concentration-dependent manner in the acrolein-phenylalanine model; and as phenylalanine decreased, a major new product eluting later than phenylalanine was generated (Fig. 3B). Importantly, the amount of the newly formed product increased with the increased amount of acrolein (Fig. 3D). The new product was then isolated and collected by semi-preparative HPLC for ESI-MS analysis. ESI-MS analysis showed that the new product had a molecular weight (MW) of 242 (*m/z* 242.1125 $[M + H]^+$) (Fig. 3C), corresponding to the molecular weight of one molecule of phenylalanine plus two molecules of acrolein after elimination of two molecules of water. Collision induced dissociation (CID) of the *m/z* 242 analytes gave *m/z* 94 fragment ion (Fig. 3C), probably corresponding to a 3-methylpyridinium moiety (Furuhata, Ishii, Kumazawa, Yamada, Nakayama & Uchida, 2003; Globisch, Deuber, & Henle, 2016). We suggested that the new product was formed in the same way as the reaction product formed between lysine and acrolein (Furuhata, Ishii, Kumazawa, Yamada, Nakayama & Uchida, 2003; Globisch, Deuber, & Henle, 2016) as follows: a nucleophilic attack of the amino group in phenylalanine at the C_1 of acrolein lead to the formation of Schiff' base that further reacted with another molecule of acrolein via Michael addition, followed by oxidation, dehydration and intramolecular cyclization to form the 3-methylpyridinium moiety.

The addition of acrolein also decreased the content of creatinine in a concentration-dependent manner in the acrolein-creatinine model (Fig. 4A); and as creatinine decreased, a major new product eluted later than creatinine was generated (Fig. 4B). Importantly, the amount of the newly formed product increased with the increased amount of acrolein (Fig. 4D). The new product was then isolated and collected by semi-preparative HPLC for ESI-MS analysis. ESI-MS analysis showed that

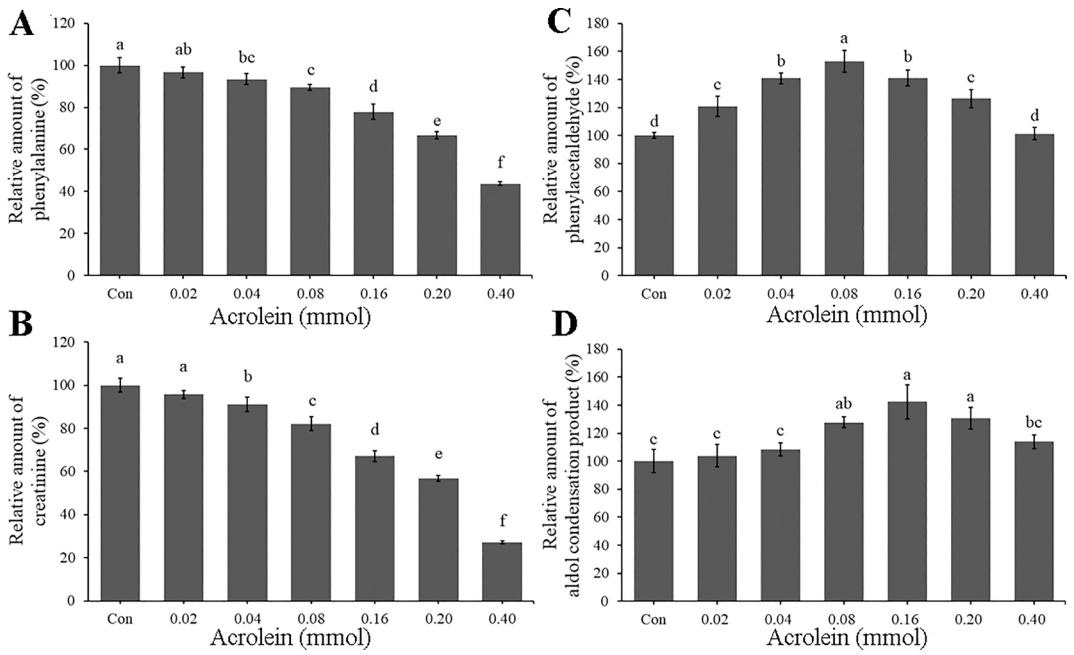

Fig. 2. Effects of different concentration of acrolein on the content of the precursors (A) phenylalanine and (B) creatinine and the key intermediates (C) phenylacetaldehyde and (D) aldol condensation product of PhIP in the PhIP-producing chemical model. Their content was determined as described in section 2.3. Values are mean ± standard deviation, n = 3. Different letters (a-f) within a column indicate significant differences among groups ($P < 0.05$).

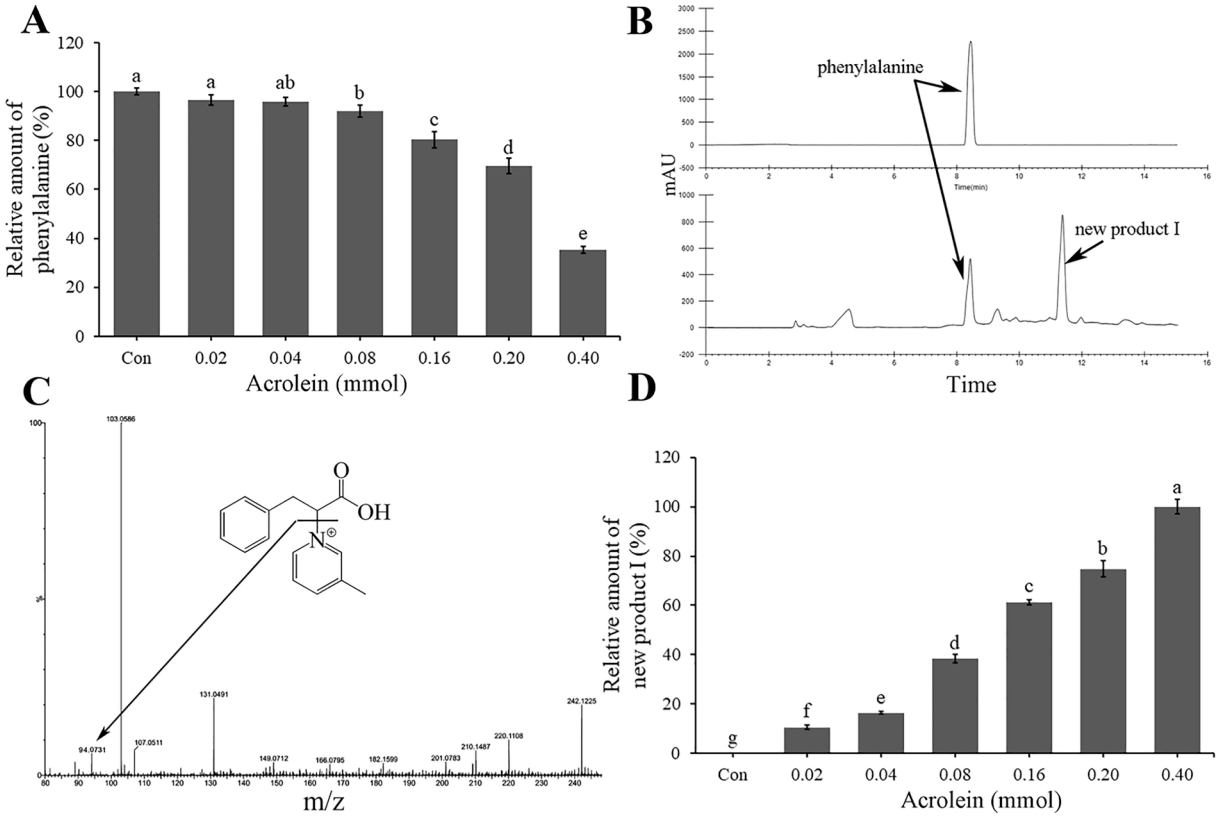

Fig. 3. Reaction between acrolein and phenylalanine in the acrolein-phenylalanine model. The model systems were set as described in section 2.4. (A) The content of phenylalanine in the acrolein-phenylalanine model. (B) HPLC profile of the reaction products between acrolein and phenylalanine in the acrolein-phenylalanine model. (C) Product ion mass spectra of the new product in the acrolein-phenylalanine model. (D) Relative amount of the new product formed in the acrolein-phenylalanine model. Values are mean ± standard deviation, n = 3. Different letters (a-g) within a column indicate significant differences among groups ($P < 0.05$).

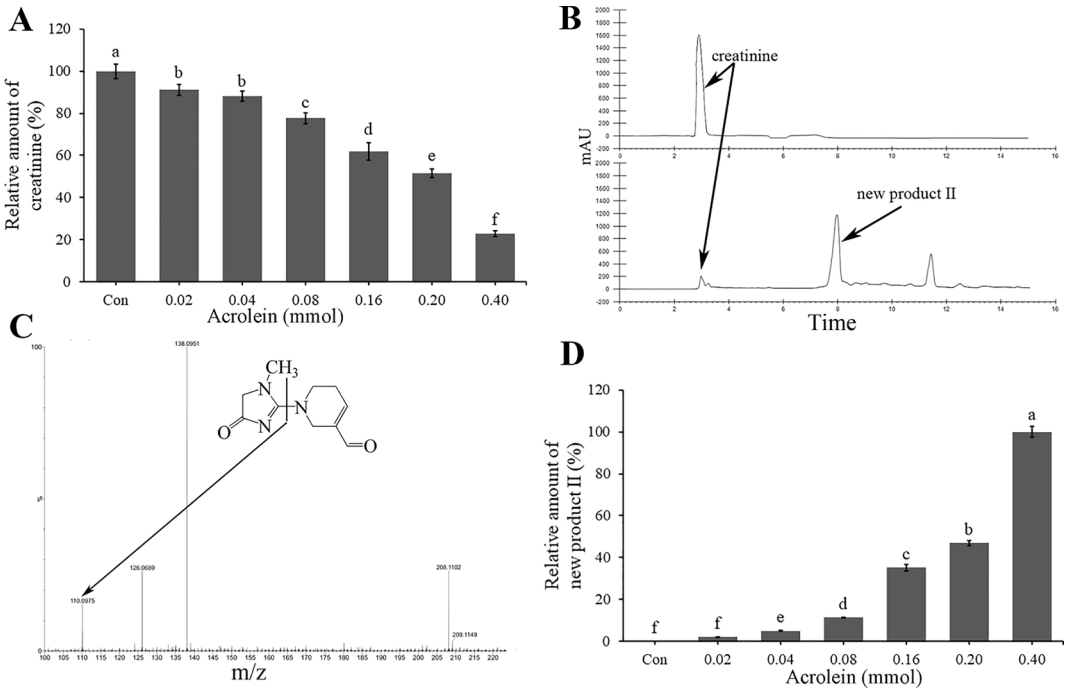

Fig. 4. Reaction between acrolein and creatinine in the acrolein-creatinine model. The model systems were set as described in section 2.4. (A) The content of creatinine in the acrolein-creatinine model. (B) HPLC profile of the reaction products between acrolein and creatinine in the acrolein-creatinine model. (C) Product ion mass spectra of the new product in the acrolein-creatinine model. (D) Relative amount of the new product formed in the acrolein-creatinine model. Values are mean ± standard deviation, n = 3. Different letters (a-f) within a column indicate significant differences among groups ($P < 0.05$).

the new product had a molecular weight (MW) of 207 (*m/z* 208.1102 $[M + H]^+$) (Fig. 4C), which was the total molecular weight of two molecules of acrolein plus one molecule of creatinine after dehydration. Collision induced dissociation (CID) of the *m/z* 208 analytes gave *m/z* 110 fragment ion (Fig. 4C), probably corresponding to a formyl-dehydropiperidino ring (Jiang et al., 2020). Therefore, we suggested that the new product was formed as follows: the amino group in creatinine reacted with the vinyl group of two molecules of acrolein via Michael addition reaction to form a di-acrolein-creatinine adduct, followed by aldol condensation of the two aldehyde groups in the di-acrolein-creatinine to form the formyl-dehydropiperidino ring.

The reaction between acrolein and PhIP was evaluated in the acrolein-PhIP model. As shown in Fig. 3H, the addition of acrolein decreased the content of PhIP in a concentration-dependent manner in the acrolein-PhIP model. A previous study found that the carbonyl group of acrolein can react with the amino group of PhIP to form a stable compound 7-hydroxyl-5-methyl-3-phenyl-5,7,8,9-tetrahydropyrido [3′,2′:4,5]imidazo[1,2-a]pyrimidine (denoted as PhIP-acrolein) (Engels

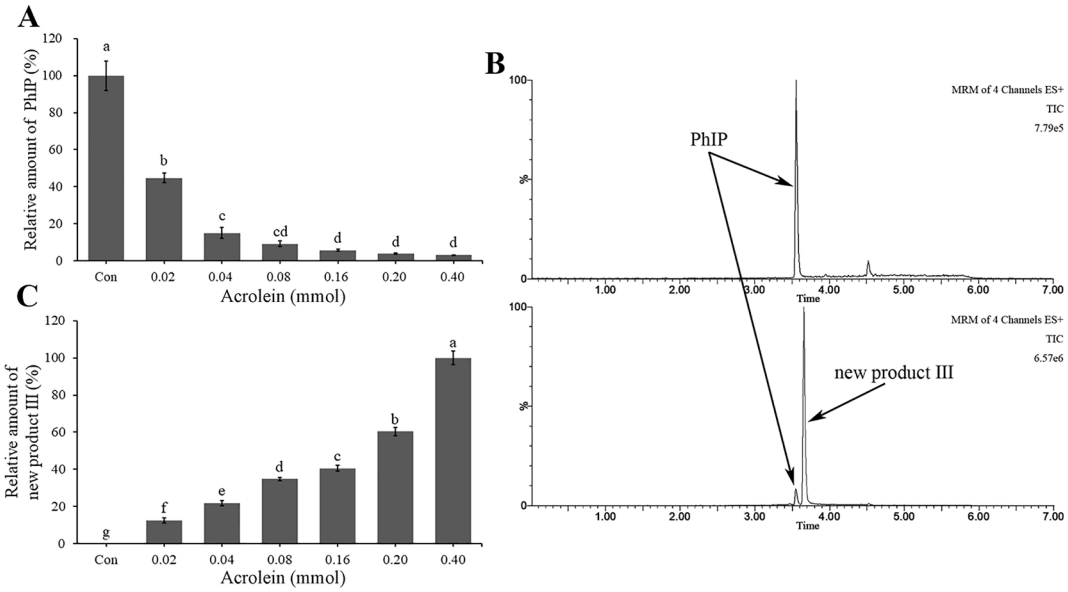

Fig. 5. Reaction between acrolein and PhIP in the acrolein-PhIP model. The model systems were set as described in section 2.5. (A) The content of PhIP in the acrolein-PhIP model. (B) HPLC profile of PhIP standard and PhIP-acrolein adduct detected by MRM (*m/z* 281/263 and *m/z* 281/225 were selected for PhIP-acrolein adduct determination; *m/z* 225.0, 210.0, and 140.0 were used for PhIP determination). (C) Relative amount of the PhIP-acrolein adduct formed in the acrolein- PhIP model. Values are mean ± standard deviation, n = 3. Different letters (a-g) within a column indicate significant differences among groups ($P < 0.05$).

et al., 2016; Vanhaecke et al., 2006; Zhang, 2018). Thus we checked if the newly formed product was PhIP-acrolein by UPLC-MS/MS. As expected, the adduct formed in the acrolein-PhIP model was the same as the compound reported by (Engels et al., 2016; Vanhaecke et al., 2006; Zhang, 2018), as evidenced by the similar MS spectrum (Figure S1). *m/z* 281/263 and *m/z* 281/225 were selected for PhIP-acrolein determination (Vanhaecke et al., 2006; Zhang, 2018); *m/z* 225/210 and *m/z* 225/140 were used for PhIP determination. As shown in Fig. 5B, as PhIP consumed, PhIP-acrolein eluting later than PhIP was generated. Importantly, the amount of PhIP-acrolein increased with the increased amount of acrolein (Fig. 5C), suggesting that excessive acrolein could react with PhIP to reduce the levels of PhIP.

Collectively, acrolein was able to contribute to PhIP formation which was a consequence of the comprehensive ability of acrolein to facilitate Strecker degradation of phenylalanine and reacting with phenylalanine, creatinine, and PhIP.

Effects of lipid oxidation on the formation of PhIP in roasted tilapia fish patties

The presence of polyunsaturated fatty acids makes the tilapia fish prone to lipid oxidation, especially during transportation and long-term storage (Karami, Moradi, Motallebi, Hosseini & Soltani, 2013; Otero et al., 2021). We next assessed the effect of lipid oxidation on PhIP formation in roasted tilapia fish patties. Aldehydes determination observed 11 aldehydes in the minced tilapia fish, among them, formaldehyde, acetaldehyde, butanal, benzaldehyde, hexanal, malondialdehyde and acrolein were the predominant species, which was in agreement with a previous study (Table S1), where similar types aldehydes were detected in tilapia fish (Shi et al., 2018). The concentration of each aldehyde in tilapia fish first increased and then decreased with increased oxidation time, which was in agreement with a previous study (Xu et al., 2014). TBARS value is an index frequently used as a marker of lipid oxidation (Soladoye et al., 2017; Zhao, Kong, Zhang, Hu, & Wang, 2019). The TBARS value of minced fish samples increased gradually during storage. When the fish samples were oxidized for 3 d (its TBARS increased from 0.33 ± 0.01 to 0.81 ± 0.03 mg MDA/kg fish), the PhIP formed in the corresponding roast fish patties increased with oxidized time, by 125% at the 3 d. Surprisingly, further oxidation of the minced fish to 9 d continued increasing the TBARS value of the fish (its TBARS increased to 1.84 ± 0.03 mg MDA/kg fish), but decreased the PhIP produced in the corresponding roast fish patties (Fig. 6). These results suggest that slight to medium oxidized tilapia fish were able to contribute to PhIP formation, while highly oxidized fish, which are unacceptable from an organoleptic point of view, do not seem to further contribute to the formation of PhIP. Moreover, these results were in line with the data obtained from the PhIP-producing chemical model where slight to medium amount of acrolein was found to contribute to PhIP formation, while high amount of acrolein did not contribute to the formation of PhIP. Collectively, these results supported the potential contribution role of lipid oxidation products to the formation of PhIP. Therefore, the control and minimization of lipid oxidation in meat and fish during transport and storage by different strategies, such as low storage temperature, vacuum packaging, modified atmosphere packaging, and addition of antioxidants could be efficient ways to inhibit HAAs formation in thermally processed meat and fish-products.

Conclusions

The effects of acrolein on PhIP formation were investigated in a PhIP-producing chemical model. It was found that acrolein increased PhIP formation at each tested addition level; 0–0.2 mmol of acrolein increased PhIP formation dose-dependently while high levels of acrolein (greater than0.2 mmol) did not further increase PhIP formation. Acrolein addition decreased the residue of phenylalanine and creatinine, but increased the content of key intermediates including phenylacetaldehyde and aldol condensation product in the PhIP-producing chemical model. Further analysis indicated that acrolein could react with phenylalanine, creatinine, and PhIP to form stable adducts. In addition, slight to medium oxidized tilapia fish contributed to PhIP formation, while highly oxidized fish did not further contribute to the formation of PhIP. Collectively, acrolein was able to contribute to PhIP formation which was a consequence of the comprehensive ability of acrolein to facilitate Strecker degradation of phenylalanine and reacting with phenylalanine, creatinine, and PhIP.

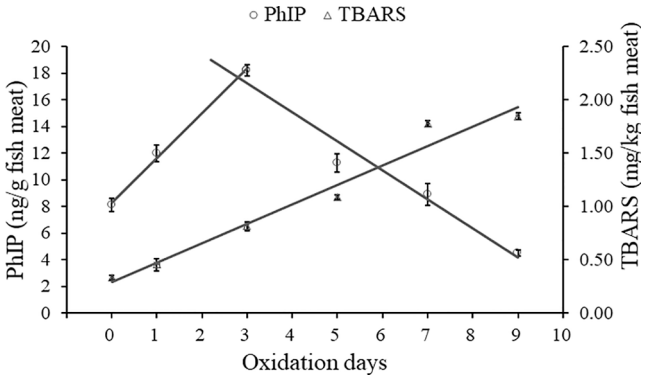

Fig. 6. Effects of oxidation time on both the formation of TBARS in raw tilapi fish (Δ) and the formation of PhIP (○) when the oxidized tilapi fish patties were roasted in an oven at 230 °C for 5 min on each side as described in section 2.7.

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.

Acknowledgements

This work was financially supported by the “National Natural Science Foundation of China (31871901 & 31901807)”, “Shanghai Science and Technology Young Talents Sailing Program (19YF1419600)”, “Shanghai Young Oriental Scholar Program (QD2019033)”, “Special Fund for Science and Technology Development of Shanghai Ocean University”, and “Startup Foundation for PhD of Shanghai Ocean University”.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100315.

References

Alaejos, M. S., & Afonso, A. M. (2011). Factors that affect the content of heterocyclic aromatic amines in foods. *Comprehensive Reviews in Food Science and Food Safety, 10* (2), 52–108.

Cao, H., Chen, B.-H., Inbaraj, B. S., Chen, L., Alvarez-Rivera, G., Cifuentes, A., … Xiao, J. (2020). Preventive potential and mechanism of dietary polyphenols on the formation of heterocyclic aromatic amines. *Food Frontiers, 1*(2), 134–151.

Dong, H., Xian, Y., Li, H., Bai, W., & Zeng, X. (2020). Potential carcinogenic heterocyclic aromatic amines (HAAs) in foodstuffs: Formation, extraction, analytical methods, and mitigation strategies. *Comprehensive Reviews in Food Science and Food safety, 19* (2), 365–404.

Engels, C., Schwab, C., Zhang, J., Stevens, M. J. A., Bieri, C., Ebert, M.-O., … Lacroix, C. (2016). Acrolein contributes strongly to antimicrobial and heterocyclic amine transformation activities of reuterin. *Scientific Reports, 6*(1). https://doi.org/10.1038/srep36246

Estévez, M., Ventanas, S., & Heinonen, M. (2011). Formation of Strecker aldehydes between protein carbonyls-α-aminoadipic and γ-glutamic semialdehydes–and leucine and isoleucine. *Food Chemistry, 128*(4), 1051–1057.

Fan, D., Li, L., Zhang, N., Zhao, Y., Cheng, K.-W., Yan, B., … Zhang, H. (2018). A comparison of mutagenic PhIP and beneficial 8-C-(E-phenylethenyl) quercetin and

6-C-(E-phenylethenyl) quercetin formation under microwave and conventional heating. *Food & Function, 9*(7), 3853–3859.
Furuhata, A., Ishii, T., Kumazawa, S., Yamada, T., Nakayama, T., & Uchida, K. (2003). Nε-(3-Methylpyridinium) lysine, a major antigenic adduct generated in acrolein-modified protein. *Journal of Biological Chemistry, 278*(49), 48658–48665.
Globisch, M., Deuber, M., & Henle, T. (2016). Identification and quantitation of the lipation product 2-amino-6-(3-methylpyridin-1-ium-1-yl) hexanoic acid (MP-Lysine) in peanuts. *Journal of Agricultural and Food Chemistry, 64*(34), 6605–6612.
Han, Z., Liu, B., Niu, Z., Zhang, Y., Gao, J., Shi, L., … Wang, S. (2017). Role of α-dicarbonyl compounds in the inhibition effect of reducing sugars on the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. *Journal of Agricultural and Food Chemistry, 65*(46), 10084–10092.
Iarc, I. A. R. C. (1993). *Some naturally occurring aromatic amines and mycotoxins*. Lyon: International Agency for Research on Cancer.
Iglesias, J., Gallardo, J. M., & Medina, I. (2010). Determination of carbonyl compounds in fish species samples with solid-phase microextraction with on-fibre derivatization. *Food Chemistry, 123*(3), 771–778.
Jägerstad, M., Skog, K., Arvidsson, P., & Solyakov, A. (1998). Chemistry, formation and occurrence of genotoxic heterocyclic amines identified in model systems and cooked foods. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A, 207*(6), 419–427.
Jiang, K., Yin, Z., Zhou, P., Guo, H., Huang, C., Zhang, G., … Ou, J. (2020). The scavenging capacity of γ-aminobutyric acid for acrolein and the cytotoxicity of the formed adduct. *Food & Function, 11*(9), 7736–7747.
Karami, B., Moradi, Y., Motallebi, A. A., Hosseini, E., & Soltani, M. (2013). Effects of frozen storage on fatty acids profile, chemical quality indices and sensory properties of red tilapia (Oreochromis niloticus × Tilapia mosambicus) fillets. *Iranian Journal of Fisheries Sciences, 12*, 378–388.
Li, Y., Fan, D., Zhao, Y., & Wang, M. (2020). Effects of quercetin and cinnamaldehyde on the nutrient release from beef into soup during stewing process. *LWT, 131*, 109712. https://doi.org/10.1016/j.lwt.2020.109712
Liu, X., Xia, B., Hu, L.-T., Ni, Z.-J., Thakur, K., & Wei, Z.-J. (2020). Maillard conjugates and their potential in food and nutritional industries: A review. *Food Frontiers, 1*(4), 382–397.
Liu, S., Zhu, Y., Liu, N., Fan, D., Wang, M., & Zhao, Y. (2021). Antioxidative properties and chemical changes of quercetin in fish oil: Quercetin reacts with free fatty acids to form its ester derivatives. *Journal of Agricultural and Food Chemistry, 69*(3), 1057–1067.
Ni, W., McNaughton, L., LeMaster, D. M., Sinha, R., & Turesky, R. J. (2008). Quantitation of 13 heterocyclic aromatic amines in cooked beef, pork, and chicken by liquid chromatography-electrospray ionization/tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 56*(1), 68–78.
Otero, P., Carpena, M., Fraga-Corral, M., Garcia-Oliveira, P., Soria-Lopez, A., Barba, F., … Prieto, M. (2021). Aquaculture and agriculture-by products as sustainable sources of omega-3 fatty acids in the food industry. *eFood, 2*(5), 209–233.
Shi, C.e., Yang, X., Han, S., Fan, B., Zhao, Z., Wu, X., & Qian, J. (2018). Nondestructive prediction of tilapia fillet freshness during storage at different temperatures by integrating an electronic nose and tongue with radial basis function neural networks. *Food and Bioprocess Technology, 11*(10), 1840–1852.
Soladoye, O. P., Shand, P., Dugan, M. E. R., Gariépy, C., Aalhus, J. L., Estévez, M., & Juárez, M. (2017). Influence of cooking methods and storage time on lipid and protein oxidation and heterocyclic aromatic amines production in bacon. *Food Research International, 99*, 660–669.
Vanhaecke, L., Van Hoof, N., Van Brabandt, W., Soenen, B., Heyerick, A., De Kimpe, N., … Van de Wiele, T. (2006). Metabolism of the food-associated carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by human intestinal microbiota. *Journal of Agricultural and Food Chemistry, 54*(9), 3454–3461.
Wang, R.u., Tao, M., Zhu, Y., Fan, D., Wang, M., & Zhao, Y. (2021). Puerarin inhibited 3-chloropropane-1, 2-diol fatty acid esters formation by reacting with glycidol and glycidyl esters. *Food Chemistry, 358*, 129843. https://doi.org/10.1016/j.foodchem.2021.129843
Wijemanne, N., Soysa, P., Wijesundara, S., & Perera, H. (2018). Development and validation of a simple high performance liquid chromatography/UV method for simultaneous determination of urinary uric acid, hypoxanthine, and creatinine in human urine. *International Journal of Analytical Chemistry, 2018*, 1–6.
Xu, Y., Li, H., Liang, J., Ma, J., Yang, J., Zhao, X., … Dong, H. (2021). High-throughput quantification of eighteen heterocyclic aromatic amines in roasted and pan-fried meat on the basis of high performance liquid chromatography-quadrupole-orbitrap high resolution mass spectrometry. *Food Chemistry, 361*, 130147. https://doi.org/10.1016/j.foodchem.2021.130147
Xu, Y., Liu, Y., Jiang, C., Zhang, C., Li, X., Zhu, D., & Li, J. (2014). Determination of volatile compounds in turbot (*Psetta maxima*) during refrigerated storage by headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Journal of the Science of Food and Agriculture, 94*(12), 2464–2471.
Xu, X., Xue, T., Jiang, Q., Fan, D., Wang, M., & Zhao, Y. (2022). Inhibitory effects of some hydrocolloids on the formation of N^{ε}-(carboxymethyl) lysine and N^{ε}-(carboxyethyl) lysine in chemical models and fish patties. *LWT-Food Science and Technology, 162*, Article 113431.
Yang, H., Ji, Z., Wang, R.u., Fan, D., Zhao, Y., & Wang, M. (2021). Inhibitory effect of selected hydrocolloids on 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) formation in chemical models and beef patties. *Journal of Hazardous Materials, 402*, 123486. https://doi.org/10.1016/j.jhazmat.2020.123486
Yu, D.i., & Yu, S.-J. (2016). Effects of waxy rice starch and short chain amylose (SCA) on the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in a model system. *Food & Function, 7*(3), 1339–1344.
Zamora, R., Alcón, E., & Hidalgo, F. J. (2012). Effect of lipid oxidation products on the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in model systems. *Food Chemistry, 135*(4), 2569–2574.
Zamora, R., Delgado, R. M., & Hidalgo, F. J. (2012). Chemical conversion of phenylethylamine into phenylacetaldehyde by carbonyl-amine reactions in model systems. *Journal of Agricultural and Food Chemistry, 60*(21), 5491–5496.
Zamora, R., & Hidalgo, F. J. (2015). 2-Amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP) formation and fate: An example of the coordinate contribution of lipid oxidation and Maillard reaction to the production and elimination of processing-related food toxicants. *RSC Advances, 5*(13), 9709–9721.
Zamora, R., & Hidalgo, F. J. (2016). The triple defensive barrier of phenolic compounds against the lipid oxidation-induced damage in food products. *Trends in Food Science & Technology, 54*, 165–174.
Zhang, J. (2018). Impact of gut microbiota on the metabolism of carcinogenic dietary heterocyclic amines (Doctoral dissertation, ETH Zurich).
Zhang, X.u., Ni, L., Zhu, Y., Liu, N., Fan, D., Wang, M., & Zhao, Y. (2021). Quercetin inhibited the formation of lipid oxidation products in thermally treated soybean oil by trapping intermediates. *Journal of Agricultural and Food Chemistry, 69*(11), 3479–3488.
Zhao, Y., Kong, H., Zhang, X.u., Hu, X., & Wang, M. (2019). The effect of Perilla *(Perilla frutescens*) leaf extracts on the quality of surimi fish balls. *Food Science & Nutrition, 7*(6), 2083–2090.
Zhao, Y., Yang, H., Zhang, N., Zhou, Q., Fan, D., & Wang, M. (2021). Effects of the deacetylation degree of chitosan on 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP) Formation in chemical models and beef patties. *Journal of Agricultural and Food Chemistry, 69*(46), 13933–13941.
Zhao, Y., Zhang, X.u., Zhang, N., Zhou, Q., Fan, D., & Wang, M. (2022). Lipophilized apigenin derivatives produced during the frying process as novel antioxidants. *Food Chemistry, 379*, 132178. https://doi.org/10.1016/j.foodchem.2022.132178
Zhu, Q., Zheng, Z.-P., Cheng, K.-W., Wu, J.-J., Zhang, S., Tang, Y. S., … Wang, M. (2009). Natural polyphenols as direct trapping agents of lipid peroxidation-derived acrolein and 4-hydroxy-trans-2-nonenal. *Chemical Research in Toxicology, 22*(10), 1721–1727.
Zöchling, S., & Murkovic, M. (2002). Formation of the heterocyclic aromatic amine PhIP: Identification of precursors and intermediates. *Food Chemistry, 79*(1), 125–134.