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Effects of deep fat frying conditions on the formation of heterocyclic aromatic amines in chicken meat

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Abstract

Deep fat frying is a common cooking procedure; however, its effects on food can produce carcinogenic compounds, namely heterocyclic aromatic amines (HAAs) that are detrimental to the health of the consumer. The influence of frying process conditions (temperature, time, meat shape, oil type, oil oxidation) on the formation of HAAs in deep fat fried chicken meat was explored. The concentrations of HAAs increased with frying temperature and time, and higher amounts were found in samples with higher surface areas. The type of oil influenced the formation of HAAs, and more HAAs were found in the samples fried in peanut and rapeseed oils. Oxidation of frying oil also elevated HAAs in fried meat. Understanding how to reduce the formation of HAAs in deep fat frying is essential for reducing the health risks associated with consuming HAAs.

Keywords: heterocyclic aromatic amines; deep fat frying; frying oil; oil oxidation; control.

Practical Application: The control of HAAs is an important way to guarantee the safety of food that has been deep fried. In order to reduce exposure to HAAs in deep fat fried meats, the raw meat and frying oil should be carefully selected, and temperature and time should be strictly controlled. The contact area between raw meat and oil should be reduced as far as possible. Frequency of use of frying oil should be reduced, and highly oxidized oil should be discarded.

1 Introduction

Frying is one of the oldest and most popular cooking methods, widely used in household kitchens and restaurants, particularly fast food restaurants, and by the food industry. Fried products are enjoyed because of their unique organoleptic properties, including crisp texture, attractive flavour, and golden color (Zhao et al., 2021; Wu et al., 2019). These unique sensory properties make fried foods popular worldwide, and sales has grown steadily and rapidly (Wu et al., 2019; Gibis, 2016; Zhao et al., 2021).

However, fried foods are among the 2A carcinogens on the draft of a carcinogen list published by the International Agency for Research on Cancer in 2017. This carcinogenicity is closely related to the content of heterocyclic aromatic amines (HAAs) (Jamali et al., 2016; Lu et al., 2018). HAAs are produced during high-temperature processing of protein-rich foods, particularly meats (Skog & Solyakov, 2002; Wang et al., 2015). The type and amount of HAAs produced depends on the following factors: meat type, cooking conditions (time, temperature, equipment, and method), pH, water content, any additives to the meats (particularly antioxidants), and, for deep fat frying, the quality of the fat (Johansson & Jägerstad, 1993; Barzegar et al., 2019; Li et al., 2021).

Deep fat fried foods are more widely consumed in greater quantities than meats prepared with other thermal treatments, such as by barbecuing, grilling, and roasting, so the effect of harmful HAAs in fried meats on health is greater. During deep fat frying, frying conditions influence many characteristics of the finished product such as oil content, flavor, texture, storage life, and nutrition, including harmful substance content (Barbut, 2013; Dunford, 2010). The conditions that produce the best quality product in terms of consumer appeal may not be the conditions that produce the healthiest product. Furthermore, unlike other cooking procedures, the quality of the cooking medium—namely, the oil—is of critical importance. The frying oil is absorbed by and it accumulates on the surface of the food; both participate in the formation of HAAs (Barbut, 2013; Dunford, 2010). The frying oil absorbed could affect HAA formation in the finished products (Johansson & Jägerstad, 1993; Ekiz & Oz, 2019). Oxidation (Johansson et al., 1995; Li et al., 2021; Zamora et al., 2012) and antioxidants (Lu et al., 2017; Gibis, 2016) in frying oil also could influence the formation of HAAs.

The production of HAAs in fried chicken is of particular importance because fried chicken is one of the most widely consumed fried foods in the world. Despite this importance, relatively few research studies have evaluated the effects of frying condition on HAA formation in deep fat fried meats, much less in chicken particularly. Therefore, this study examines the effect of deep fat frying conditions (temperature, time, meat shape, oil type, oil oxidation) on the formation of HAAs in chicken.

2 Materials and methods

2.1 Chemicals and materials

Materials

Soybean oil, palm liquid oil, sunflower seed oil, rapeseed oil, peanut oil, and raw chicken breast were purchased from

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local supermarkets for deep-fat frying experiments. Lard was provided by Tianjin jiuyuan oil & fats CO., LTD.

Chemicals

Harman and Norharman were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) and other HAAs were obtained from Toronto Research Chemicals Inc. (Toronto, Canada). Purity of HAA standards were between 98.0% and 99.9%.

2.2 Preparation of deep-fat fried chicken meat

Preparation of raw materials

Trimmed bone and fat layer from raw chicken breast, and cut it into small sections, and then used multifunctional food mincing machine (Joyoung JYL-C022E, China) to make chicken minces. Preparation of patties, nuggets, and meatballs referenced Supplementary data, Table S3 and Figure S2. All raw materials were put in a resealable bag for deep-fat frying experiments.

Deep-fat frying

1.0L frying oils were added into the Weightmax XJ-6K116 fryer (20.5 × 12.0 × 10.5 cm, volume 1.5 L) (Hubei Xiangjiang Electric Appliance Co., Ltd, Hubei, China). Raw materials prepared were put into fryer according to the oil-meat mass ratio of 10:1 after oil reached the setting frying temperature. And they were dipped into hot oil for a certain time. Different with roasting, frying, and boiling, deep-fat frying was a special cooking technology using oil as heating medium. Temperatures of frying oil would decrease because of the existence of raw materials and fluctuate as cooking the raw materials. Because the temperature directly affected the formation of HAAs, the monitor for real-time oil temperature (160 °C, 180 °C, 200 °C, and 220 °C) was performed by intelligent temperature controller (Henan Gongyi Yuhua Instrument Equipment Co., Ltd, China) and shown in Supplementary data, Figure S1. The change occurred was consistent with the four stages happening in deep-fat frying process: initial heating, surface boiling, falling rate, and bubble end-point (Ni & Datta, 1999). Then, the deep-fat fried chicken meats were prepared and put in a filter for 5 minutes in order to cool to room temperature. Blot papers were used to absorb the excess oil from surface. Samples were crushed and sifted by a 30-mesh sieve, and then store them in resealable bags at -20 °C temperature for analysis.

2.3 Assay of HAAs

The extraction and determination of HAAs in frying oils and deep-fat fried chicken meats was performed as described by the Zhang et al. (2020b). Cleanert PCX cartridges (3 mL/60 mg), obtained from Agela Technologies (Tianjin, China), were used for solid-phase extraction. Separation process was implemented through Waters ACQUITY UPLC BEH C18 column (1.7 μm, 50 mm x 2.1 mm i.d) (Waters, Milford, MA, USA) and the detection was completed by Waters Acquity UPLC CLASS system (Waters, Milford, MA, USA) and Triple quadrupole mass spectrometry (Xevo TQD) with a positive electrospray ionisation (ESI +) (Waters, Milford, MA, USA).

2.4 Statistical analysis

The HAAs chromatograph was processed through Waters MassLynx 4.1 software (Waters, Milford, MA, USA). All statistics were conducted with the IBM SPSS software package version 21 (SPSS Inc., Chicago, USA). All data are the average of three determinations.

3 Results and discussion

3.1 HAAs in raw chicken meat and frying oils

The seven types of HAAs assessed in this study of raw chicken meat and frying oils are shown in Table 1.

It can be seen that none of the seven were detected in raw chicken, and similar results have been found in other studies (Ekiz & Oz, 2019; Yang et al., 2016). None of the seven were detected in lard, palm liquid oil, sunflower seed oil and soybean oil, while norharman (2.40 ng/g and 3.07 ng/g) and harman (1.06 ng/g and 1.88 ng/g) were detected in rapeseed oil and peanut oil. This was consistent with the research of Chang et al. (2019). They found norharman and harman in peanut oil in concentrations of 63 pmol/g and 39 pmol/g, respectively, while several other research studies did not find HAAs in soybean oil, palm liquid oil, or sunflower seed oil. We speculate that the high concentrations of norharman and harman occur in rapeseed and peanut oils because these HAAs are easily produced from the degradation of tryptophan during roasting of the seeds to produce the oils (Herraiz, 2004; Zhang et al., 2020a).

3.2 Effect of temperature and time on the formation of HAAs

Soybean oil is the common choice in restaurants and home kitchens for deep-frying, because it is relatively tasteless, odorless,

Table 1. The concentrations of HAAs in raw chicken meat and frying oils (ng/g).

Materials	IQ	MeIQ	MeIQx	4,8-DiMeIQx	PhIP	Harman	Norharman
Raw Chicken	nd	nd	nd	nd	nq	nd	nd
Lard Oil	nd	nd	nd	nd	nd	nd	nd
Palm Liquid Oil	nd	nd	nd	nq	nd	nd	nq
Sunflower Seed Oil	nd	nd	nd	nq	nd	nd	nq
Soybean Oil	nd	nd	nd	nq	nd	nd	nq
Rapeseed Oil	nd	nd	nd	nd	nq	1.06 ± 0.08	2.40 ± 0.09
Peanut Oil	nd	nd	nd	nd	nq	1.88 ± 0.09	3.07 ± 0.10

nd: not detected; nq: not quantified.

inexpensive, with a relatively high smoke point. Therefore, soybean oil was chosen for all subsequent experiments. It is well known that temperature and time are two of the most important factors affecting the formation of HAAs. In order to evaluate the content and distribution of HAAs in detail, soybean oil was put in the fryer, and the frying temperature was set at 160, 180, 200 and 220 ± 2 °C, meanwhile, at each temperature, the frying time was set at 4, 6, 8, and 10 min. HAAs produced are shown in Table 2.

Two imidazoquinolines, IQ and MeIQ, have similar pyridine and imidazole ring structures, but MeIQ has one more exocyclic methyl. IQ was not detected in samples fried at 160 °C, 180 °C, and 200 °C, but was found in samples fried at 220 °C (0.27~1.00 ng/g). Similarly, MeIQ was not detected in low-temperature samples (160 °C and 180 °C), but was found at concentrations of 0.11 and 1.26 ng/g in meats fried at 200 °C and 220 °C, respectively. IQ and MeIQ have been largely undetected in most studies (Haskaraca et al., 2017; Jinap et al., 2013). However, in one study, at high temperature, thermal processing increased IQ and MeIQ (Chiu et al., 1998).

MeIQx and 4,8-DiMeIQx, two imidazoquinoxalines, both contain pyrazine and imidazole rings and are the most likely to be affected by frying temperature and time duration. In our results, the concentration of MeIQx ranged from ND to 3.10 ng/g, which was similar with the results described by Haskaraca et al. (2017). And the concentration of 4,8-DiMeIQx ranged from ND to 0.93 ng/g. The relationship between the amounts of these two imidazoquinoxalines and the processing conditions conform to the first-order kinetics model. The concentration of MeIQx was higher than 4,8-DiMeIQx under all conditions. In the research of Haskaraca et al. (2014), MeIQx was the main HAA in fried chicken, while 4,8-DiMeIQx was not detected. Similar results were obtained by Ekiz & Oz (2019). Arvidsson et al. (1997) indicated that the formation rate of MeIQx was higher than that of 4,8-DiMeIQx at 150 °C to 200 °C. That would explain why MeIQx was always higher than 4,8-DiMeIQx in this study.

Compared with other HAAs, PhIP has been reported to occur frequently in deep fat fried meats, especially in deep fat fried chicken (Gibis, 2016; Jinap et al., 2016; Lu et al., 2018; Skog & Solyakov, 2002). Imidazopyridines are easily formed, and were detected in all samples (Table 2). PhIP increased significantly when the temperature was higher (p<0.05). Arvidsson et al.(1997) fitted a first-order reaction model and the Eyring equation to the formation of PhIP to obtain rate constants and their temperature dependence. The significant increase of PhIP may also be related to the high fat and lipid oxidation in meats and oils under high temperatures. Fat would affect the heat transfer rate, and the oil oxidations would give rise to the formation of PhIP through the Maillard reaction (Jägerstad et al., 1998; Zamora et al., 2012). Nevertheless, PhIP formation with different amounts of fat in the food still follows first-order kinetics (Hwang & Ngadi, 2002). β-carbolines mainly include harman and norharman which were easily detected in meat and fish products that were heated at high temperatures (Zhang et al., 2020c). The structure of these two HAAs is similar with that of the decomposition products of amino acids. In this study, harman and norharman were both detected in all deep fat fried chicken meats, and the concentration of norharman (0.35-8.00 ng/g) was higher than that of harman (0.12-6.76 ng/g). Harman and norharman were the lowest in samples fried at 160 °C, and higher temperature significantly promoted their formation (p<0.01) (Jamali et al., 2016). Gibis et al. (2015) reported that levels of harman and norharman in bacon samples prepared at 200-220 °C were much higher than that at 150 °C-170 °C. Although decreasing with the increase of time under high temperature (130 °C to 225 °C) (Arvidsson et al., 1999; Chiu & Chen, 2000), harman and norharman steadily increased due to their high stability. It also could be seen that frying temperature had greater influence on harman and norharman production than time.

3.3 Effect of meat shape on the formation of HAAs

Three forms of raw chicken meat: patties, nuggets, and meatballs, were deep fat fried at 180 °C for 6 min. HAAs were determined (Figure 1). MeIQ, MeIQx, 4,8-DiMeIQx, PhIP, harman, and norharman (except IQ) were detected in all samples, and the total HAA content variation, from high to low, was: Fried meat patties > Fried meat nuggets > Fried meat meatballs. Fried meat meatballs had the least amount of HAAs; concentrations of MeIQ, MeIQx, 4,8-DiMeIQx, PhIP, harman, and norharman were 0.11, 0.61, 0.16, 1.60, 0.25, and 1.11 ng/g, respectively. HAAs in fried meat nuggets were 1.87, 1.24, 1.53, 1.29, 1.27, and 1.31 times of those in meatballs, and HAAs in fried meat patties 2.37, 1.48, 2.32, 1.63, 1.44, and 1.57 times of those in meatballs.

Given identical frying conditions, the amount of HAAs produced is related to the surface area of the meat exposed to oil. The correlation between HAAs and the surface area was investigated (Supplementary data, Table S3 and Figure S2), and a significant positive correlation (p<0.01) was found, with correlation coefficients in the range of 0.936 to 0.994. In other words, in the presence of precursors at high temperatures, the greater the area in contact with oil the greater the production of HAAs. Other studies have

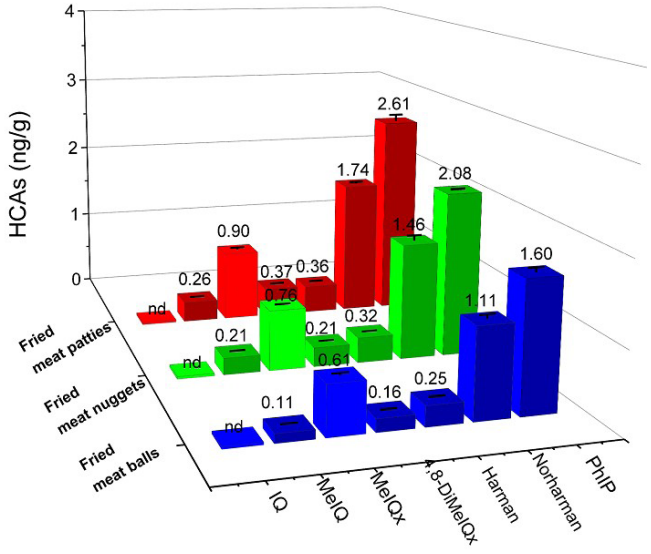

Figure 1. Effect of meat shape on the concentrations of HAAs in fried chicken (ng/g).

Table 2. Effect of temperature and time duration on HAAs in fried chicken (ng/g).

	Tep (°C)	Time (min)				F	p
		4	6	8	10		
IQ	160	nd	nd	nd	nd	-	-
	180	nd	nd	nd	nd	-	-
	200	nq	nq	nq	nq	-	-
	220	0.27 ± 0.01	0.54 ± 0.03	0.82 ± 0.03	1.00 ± 0.04	311.93	<0.001
	F	1492.63	784.38	2319.75	1684.92		
	p	<0.001	<0.001	<0.001	<0.001		
MeIQ	160	nd	nd	nd	nd	-	-
	180	nd	nd	nd	nd	-	-
	200	nq	nq	0.26 ± 0.01	0.41 ± 0.001	3085.21	<0.001
	220	0.11 ± 0.006	0.39 ± 0.005	0.78 ± 0.05	1.26 ± 0.07	353.294	<0.001
	F	1111.52	14808.81	550.78	763.58		
	p	<0.001	<0.001	<0.001	<0.001		
MeIQx	160	nd	nq	0.33 ± 0.008	0.47 ± 0.02	2590.531	<0.001
	180	0.30 ± 0.01	0.53 ± 0.02	0.71 ± 0.02	0.90 ± 0.02	674.26	<0.001
	200	0.72 ± 0.03	1.17 ± 0.02	1.59 ± 0.05	1.96 ± 0.11	236.66	<0.001
	220	1.03 ± 0.04	1.58 ± 0.08	2.23 ± 0.13	3.10 ± 0.08	322.35	<0.001
	F	1122.46	860.86	482.14	907.62		
	p	<0.001	<0.001	<0.001	<0.001		
4,8-DiMeIQx	160	nq	nq	0.11 ± 0.001	0.13 ± 0.006	1711.35	<0.001
	180	0.14 ± 0.01	0.29 ± 0.01	0.37 ± 0.006	0.42 ± 0.01	361.33	<0.001
	200	0.23 ± 0.01	0.41 ± 0.008	0.57 ± 0.02	0.70 ± 0.01	577.58	<0.001
	220	0.33 ± 0.01	0.54 ± 0.02	0.70 ± 0.009	0.93 ± 0.007	1140.75	<0.001
	F	488.58	1224.49	1181.5	3678.82		
	p	<0.001	<0.001	<0.001	<0.001		
PhIP	160	0.19 ± 0.007	0.30 ± 0.02	0.42 ± 0.03	0.50 ± 0.04	94.2	<0.001
	180	0.58 ± 0.03	1.31 ± 0.06	2.52 ± 0.02	4.20 ± 0.17	920.36	<0.001
	200	10.21 ± 0.60	13.18 ± 0.35	17.22 ± 0.70	20.84 ± 1.10	118.25	<0.001
	220	25.97 ± 0.46	34.53 ± 3.22	52.99 ± 3.13	67.92 ± 3.62	126.63	<0.001
	F	3039.1	289.45	687.74	803.78		
	p	<0.001	<0.001	<0.001	<0.001		
Harman	160	0.12 ± 0.01	0.14 ± 0.003	0.15 ± 0.004	0.17 ± 0.007	28.76	<0.001
	180	0.23 ± 0.01	0.36 ± 0.006	0.43 ± 0.03	0.53 ± 0.05	56.05	<0.001
	200	0.48 ± 0.05	0.86 ± 0.03	1.20 ± 0.06	1.58 ± 0.09	181.29	<0.001
	220	1.45 ± 0.09	3.13 ± 0.12	4.34 ± 0.16	6.76 ± 0.30	441.51	<0.001
	F	398.75	1408.41	1473.33	1160.19		
	p	<0.001	<0.001	<0.001	<0.001		
Norharman	160	0.35 ± 0.01	0.43 ± 0.02	0.46 ± 0.006	0.55 ± 0.05	30.33	<0.001
	180	0.52 ± 0.03	0.83 ± 0.04	1.08 ± 0.02	1.32 ± 0.04	311.59	<0.001
	200	1.35 ± 0.08	1.83 ± 0.04	2.35 ± 0.06	3.20 ± 0.10	635.52	<0.001
	220	2.39 ± 0.03	3.77 ± 0.09	5.14 ± 0.34	8.00 ± 0.50	231.95	<0.001
	F	1419.47	6245.02	443.3	720.28		
	p	<0.001	<0.001	<0.001	<0.001		

Tep: Temperture; F: F value, which was calculated by variance analysis; p: p value, which was calculated by variance analysis; nd: not detected; nq: not quantified.

shown that higher HAAs were associated with higher cooking loss (Knize et al., 1994), because more water-soluble precursors migrated to the surface with water in samples with the large surface area. The water-soluble precursors and frying oil absorbed was more likely to accumulate on the surface of fried food and simultaneously leaded to the formation of HAAs. The addition of components with water holding capacity could reduce the formation of HAAs (Oz et al., 2016; Vangnai et al., 2014).

3.4 Effect of frying oil type on the formation of HAAs

Six frying oils widely used in homes and restaurants-- lard, soybean oil, palm liquid oil, sunflower seed oil, rapeseed oil, and peanut oil-- were selected to investigate the effects on HAAs in deep fat fried chicken. In order to make the tests realistic, frying was carried out under 180 °C for 6 min. The oil was collected and the fryer was cleaned after each experiment. All frying was done in the same fryer. HAAs in corresponding chicken meats fried in different oils were determined (Figure 2).

IQ was not detected in any chicken meat irrespective of the frying oil, which suggested that IQ was not readily formed at frying temperatures lower than 200 °C, and this was consistent with other research results (Haskaraca et al., 2014). MeIQ had the highest concentration in CM-PO (0.20 ng/g), but it was not detected in other samples (Figure 2). Table 2 also showed that no MeIQ were detected at 180 ° C. As a result, the formation of MeIQ could be influenced by the peanut oil, especially the Maillard reaction products, such as pyrazines among it. Milić et al. (1993) used ESR, ^{1}H NMR, and MS to propose the formation mechanism of MeIQ in model system (2-methylpyridine, creatinine, and acetaldehyde).

In another study, MeIQx was the dominant HAA in samples obtained from fast food restaurants (Haskaraca et al., 2014). In this study, MeIQx was detected in all samples and significantly different for different oils (p<0.01). CM-LO had the lowest MeIQx, followed by CM-SsO, CM-SO, CM-PlO, CM-RO, and CM-PO. MeIQx levels were significantly lower than the levels of MeIQx (30.43-43.71 ng/g) detected by Ekiz & Oz (2019). The effect of type of frying oil on 4,8-DiMeIQx was similar with the effect on MeIQx. CM-LO and CM-SsO had the lowest 4,8-DiMeIQx concentrations, followed by CM-SO, CM-PlO, CM-RO, and CM-PO.

High levels of MeIQx and 4,8-DiMeIQx were detected in the meats fried in rapeseed and peanut oils. The consistency of the effect of type of frying oil on MeIQx and 4,8-DiMeIQx could be explained by how the two carcinogens are formed. The imidazole part of the imidazoquinoxalines was formed by cyclization of creatinine; and the rest of the molecule was derived from pyrazines and aldehydes. Milić et al. (1993) detected the MeIQx in reaction mixtures (2,5-dimethylpyrazine, creatinine, and acetaldehyde). Pearson et al. (1992) detected the MeIQx and 4,8-DiMeIQx in heating mixtures containing dialkyl-pyrazine radicals and creatinine. High levels of 4,8-DiMeIQx in CM-SO and CM-PO are possibly related to the high content of pyrazines and aldehydes naturally present in these two oils. Dun et al. (2019) found that peanut oil contains pyrazines (methylpyrazines and 2,5-dimethylpyrazines) and aldehydes. Similar substances were also detected in rapeseed oil (Jing et al., 2020).

Type of oil also had a significant effect on the production of PhIP (p<0.05). PhIP in CM-LO, CM-SsO, and CM-SO was 1.27, 1.29, and 1.97 ng/g, respectively. The concentration of PhIP in CM-PO was relatively high (4.12 ng/g), while PhIP was even higher in CM-RO (5.89 ng/g) and CM-PO (8.00 ng/g). The formation of PhIP was significantly influenced by constituents and derivatives of oil (Johansson et al., 1995; Li et al., 2021; Lu et al., 2017; Zamora et al., 2012).

Concentrations of harman and norharman in CM-RO and CM-PO were 1.43 and 3.59 ng/g, and 2.36 and 5.77 ng/g,

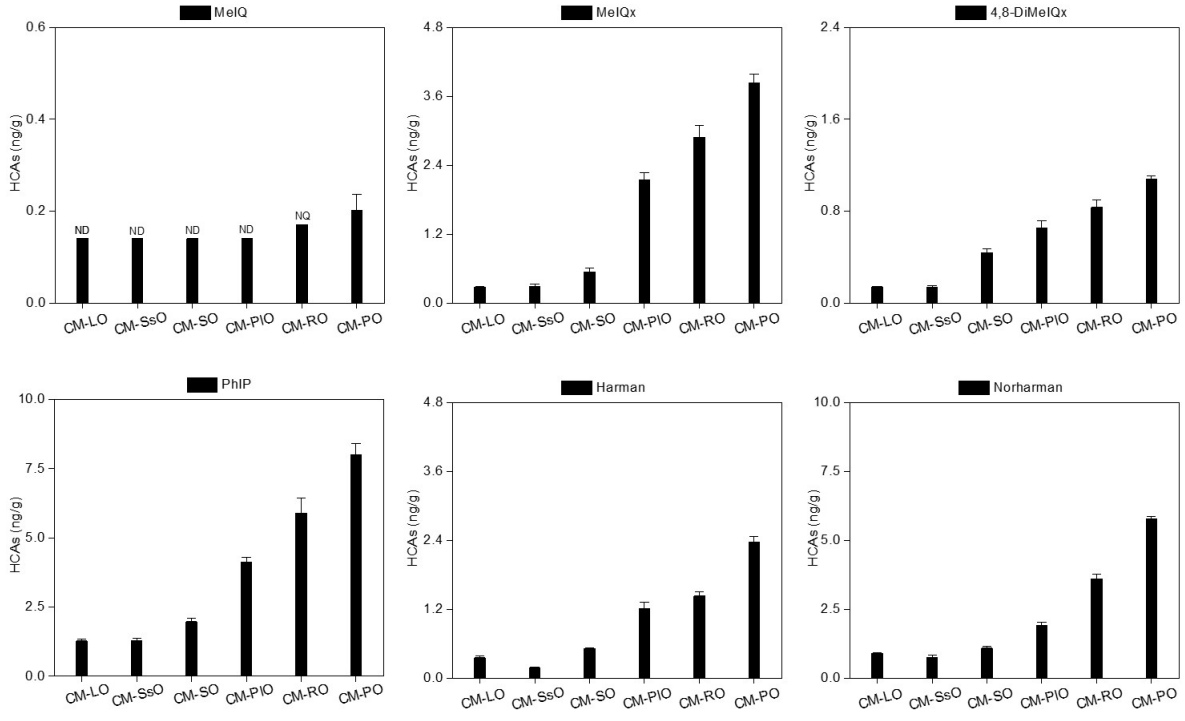

Figure 2. Effect of oil type on the concentrations of HAAs in fried chicken (ng/g). CM-LO: Chicken meats fried in lard oil; CM-SsO: Chicken meats fried in sunflower seed oil; CM-SO: Chicken meats fried in soybean oil; CM-PlO: Chicken meats fried in palm liquid oil; CM-RO: Chicken meats fried in rapeseed oil; CM-PO: Chicken meats fried in peanut oil.

respectively. One reason for the high levels of these two β-carbolines might be the migration of HAAs pre-existing in the rapeseed and peanut oils (see Table 1) to the fried products. Harman and norharman are especially prone to such migration. Tai et al. (2001) reported the effects of lard, soybean oil and coconut oil on the formation harman and norharman. They found that oil high in saturated fatty acids was more likely to form HAAs. In this study, different results were obtained. HAAs were lower in chicken fried in lard oil, which is high in saturated fatty acids. HAAs in the CM-SO and CM-PlO were relatively high, which might be due to oxidative decomposition and release of free radicals of unsaturated fatty acids during the frying process. Such degradation and free radical formation have been proved to promote the formation of HAAs (Johansson et al., 1993).

3.5 Effect of oil oxidation on the formation of HAAs

The continuous frying and intermittent frying were performed in the laboratory, simulating the practice of most industries and shops. Chicken meat was fried at 180 °C for 6 min in this part. To continuous frying, the soybean oil was uninterruptedly heated for 20 h. One batch was fried for an hour and ten batches were fried. Oil was reheated for an hour before the next batch was developed. Different with it, Oil was cooled for an hour before the next batch was developed in intermittent frying. Oil samples (Supplementary data, Table S4) were taken after each batch had been fried, and the amount of HAAs measured (Figure 3 and Figure 4). IQ (<0.03 ng/g) was not detected, which was consistent with previous studies and increase in the number of times frying oil had been used correlated with increase in the concentrations of different types of HAAs.

Continuous frying

At the same frying temperature and time, five types of HAAs were detected in the samples from first to sixth batch, while six types were detected in the seventh to tenth batches. MeIQx, 4,8-DiMeIQx, PhIP, harman, and norharman were detected in the samples from all batches. The concentration of HAAs (except IQ) increased with repeated frying. The levels of MeIQ, MeIQx, 4,8-DiMeIQx, PhIP, harman, and norharman were 0.09, 0.47, 0.13, 1.40, 0.22 and 0.82 ng/g, respectively, in the samples of the first batch. Levels of MeIQ, MeIQx, 4,8-DiMeIQx, PhIP, harman, and norharman were 0.25, 0.76, 0.35, 7.11, 1.02 and 2.18 ng/g, respectively, in the samples of the tenth batch, increasing 2.61, 1.62, 2.31, 5.07, 4.72 and 2.65 times, respectively. In contrast, Wang et al. (2015) studied the effect of frying times on the formation of HAAs in grass carp (*Ctenopharyngodon idellus*).

The increase of frying times increased the types of HAAs, but the concentration of HAAs showed no significant difference with the number of frying cycles. Among the 7 HAAs detected, oxidation of the oil has the most obvious effect on the formation of PhIP in fried meats. Zamora & Hidalgo (2015) proposed that lipid-derived carbonyl compounds can convert phenylalanine to phenylacetaldehyde through Strecker degradation, thus contributing to the formation of PhIP. Zamora et al. (2012) also showed that lipid oxidation products, such as 4-oxo-2-nonenal, promoted the formation of PhIP in a model system. The amounts of PhIP in phenylalanine/creatinine mixtures without any lipid oxidation were 7.92 pmol/μmol creatinine, but those in the same model system with 4-oxo-2-nonenal were 32.48 pmol/μmol creatinine (4.1 times). Similarly, it was found in the model system that the addition of oxidized soybean oil also increased the formation of PhIP. Presumably, the higher PhIP was related to thermal

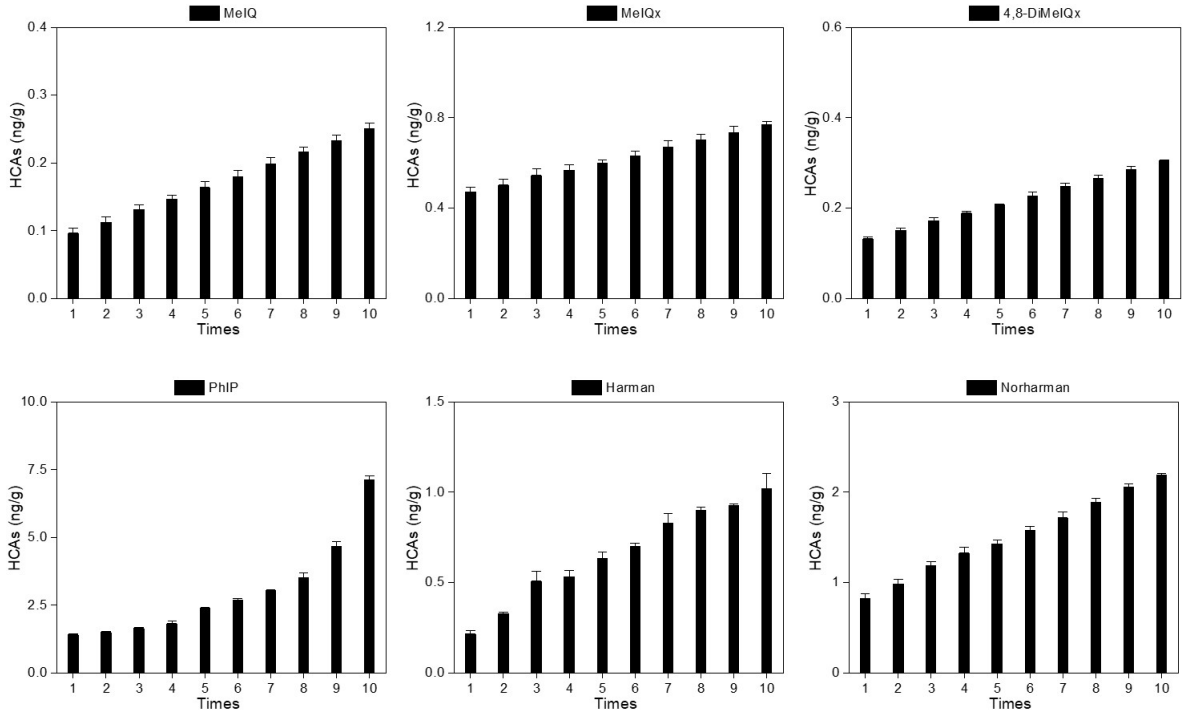

Figure 3. Effect of oxidation of oils on HAAs in fried chicken during continuous frying (ng/g).

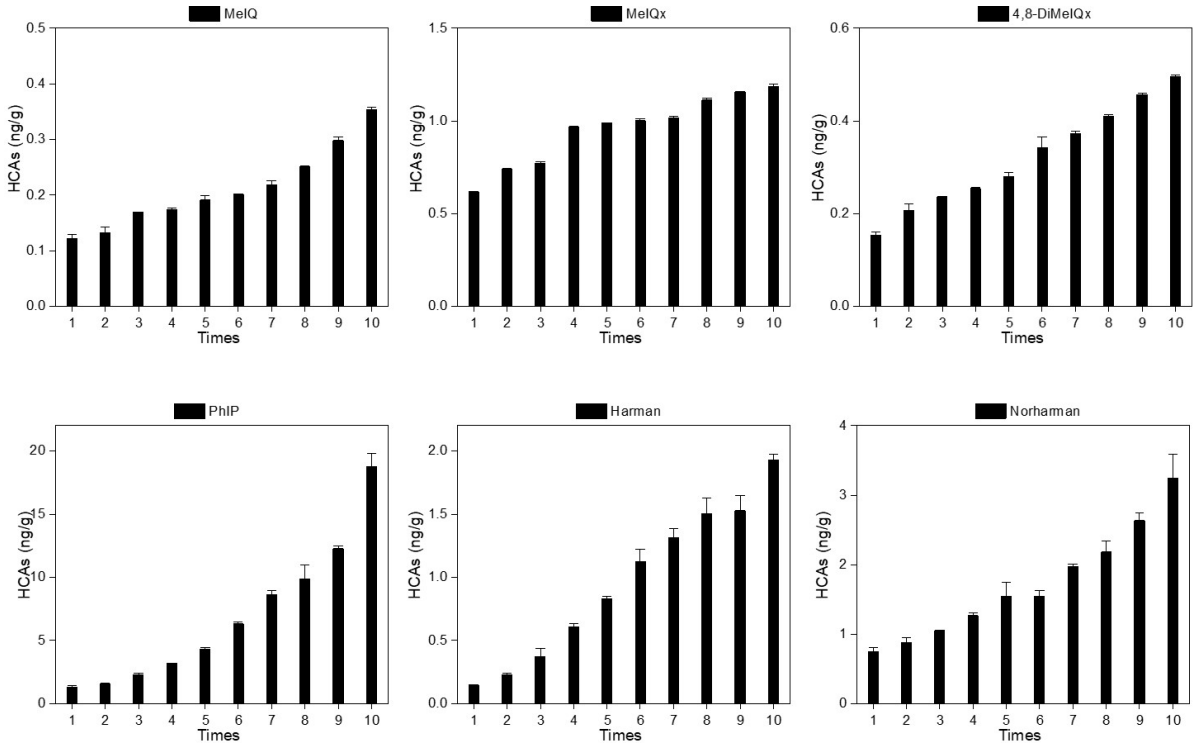

Figure 4. Effect of oxidation of oils on HAAs in fried chicken during intermittent frying (ng/g).

decomposition, oil oxidation, and the interactions between food and oil. In addition, Lu et al. (2017) indicated that PhIP was significantly reduced in pork patties after replacing lard with any of vegetable oil, olive oil, sunflower seed oil, or grape seed oil, and concluded the differences were related to the high levels of antioxidants in the vegetable oils. The results in this study, i.e., higher PhIP found in meat fried in tenth batch, were consistent with previous researches. Therefore, higher PhIP might be due to the low levels of antioxidants in oxidized oil (Supplementary data, Table S4). What is noteworthy is that when meat residues remained in the oil, more HAAs would form when the frying oil was heated and used repeatedly (Wang et al., 2015). The residues could bind to the meat surface, further increasing the HAAs. Therefore, meat residues should be removed from the oil before it is re-used for deep fat frying. Due to the different formation mechanism of each HAA and the complex components in oxidized oil, it was hard to determine the specific pathways involved.

Intermittent frying

Effect of oxidation of oils on HAAs in fried chicken during intermittent frying are shown in Figure 4. The levels of MeIQ, MeIQx, 4,8-DiMeIQx, PhIP, harman, and norharman were 0.12, 0.61, 0.15, 1.32, 0.14 and 0.75 ng/g, respectively, in the samples of the first batch. Levels of MeIQ, MeIQx, 4,8-DiMeIQx, PhIP, harman, and norharman were 0.35, 1.19, 0.49, 18.74, 1.93 and 3.24 ng/g, respectively, in the samples of the tenth batch, increasing 2.92, 1.92, 3.21, 14.14, 13.56 and 4.32 times, respectively. Overall, the amounts of HAAs fried at highly oxidated oil were higher than those in fresh oil.

Because samples were fried at the same temperature and time, the oil was the most important factor to influence the formation of HAAs in fried meat. Compared with oil used in first batch, oxidation of oil occurred in the later batch, and oxidation of oil effected the HAAs of deep fat fried meats. The oil, after prolonged heating, would undergo chemical reactions such as oxidation, and the components of the meat products, such as water, protein, and fat, transferred into the oil, in turn, would further accelerate the reactions. The lipid oxidation products and free radicals could participate in the Maillard reaction to promote and affect the formation of HAAs (Johansson et al., 1993; Johansson & Jägerstad, 1993; Zamora & Hidalgo, 2015). The antioxidants naturally present in a particular oil, such as Vitamin E (Supplementary data, Table S4), sesamol, β-carotene, polyphenols, etc., would have a certain influence on the formation of HAAs (Lu et al., 2017; Monti et al., 2001; Vitaglione et al., 2002). Using the same oil multiple times probably increases the free radicals and decreases antioxidants in the oil. In short, the type and concentration of HAAs can be reduced by decreasing the number of times a particular oil is used for deep fat frying. However, the relationship between HAAs and oxidation products in oils needs further research.

4 Conclusion

Control of HAAs during deep fat frying depends, to a certain extent, on the process conditions. HAA levels in deep fat fried chicken were significantly affected by frying temperature and time, and increased with increase of surface area (i.e., contact area of meat with oil). The type and oxidation state of the frying oil also influenced the production of HAAs in fried meat. Total HAAs were especially high in samples fried in peanut oil and

rapeseed oil. The concentrations of HAAs in samples fried in oxidized oil (i.e., oil that had been used for frying several times) were significantly higher than those in fresh oil (p <0.01). Oil oxidation could lead to high HAAs in fried meat at the same frying temperature and time. Repeated usage of frying oil promotes lipid oxidation, which in turn promotes the formation of HAAs. In order to reduce the risk of exposure to the potentially carcinogenic HAAs in deep fat fried meats, the control of the frying process in terms of time and temperature, the choice of oil, and the use of fresh oil can help guarantee the safety of deep fat fried foods.

Conflict of interest

No conflict of interest exists in the submission of this manuscript, and the manuscript has been approved by all authors for publication.

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Supplementary data

Supplementary material accompanies this paper.

Table S1. MS parameters of seven heterocyclic aromatic amines (HAAs)

Table S2. Linear equations, correlation coefficients (R2), limits of detection (LODs) and limits of quantification (LOQs) of the seven HCAs in different matrices

Table S3. Pearson correlation among raw material surface area and HAAs

Table S4. Effects of continuous and intermittent frying on the quality of the soybean oil

Figure S1. Profiles of fried oil temperature during deep fat frying under four different temperatures and at room temperature. It was found that the oil temperature was fluctuating but stable. When the raw materials were put in fryer for about one minute, the temperature of oil barely changed, and then moisture in the food moved to the surface causing the temperature of the oil to fall rapidly. As the food cooked, the oil temperature recovered to the original state. These temperature changes corresponded to the four stages of the deep fat frying process: initial heating, surface boiling, falling rate, and bubble end-point.

Figure S2. Correlation among raw material surface area and HAAs. Preparation of patties: 10.0 ± 0.1 g chicken mince was accurately weighed to make round patties. The average diameter and height of the patties, measured by vernier calipers were about 3.5 cm and 1.0 cm, respectively, and the average surface area was about 30.22 cm2 as determined by calculation. Preparation of nuggets: 10.0 ± 0.1 g chicken mince was accurately weighed, molded to make nuggets. The average length, width, and height of the nuggets as measured were about 2, 2 and 2 cm, respectively, and the calculated average surface area was about 24.00 cm2. Preparation of meatballs: 10.0 ± 0.1 g chicken mince was accurately weighed and rubbed manually to form meatballs. The average diameter was measured to be about 2.4 cm, and the average surface area was calculated to be about 18.08 cm2.

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