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Inhibitory effect of the extract from *Sonchus olearleu* on the formation of carcinogenic heterocyclic aromatic amines during the pork cooking

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ABSTRACT

The aim of this study was to assess the inhibitory effects of *Sonchus olearleu* extract on the generation of heterocyclic amines in roasted pork patties cooked by pan-frying. All samples were cooked for two different durations (45 min and 105 min) under 200 °C and 230 °C. 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinox-aline (4,8-DiMeIQx), harman, and norharman were detected and quantified. In patties cooked at 230 °C for 105 min, *S. olearleu* extract (0.5%) significantly inhibited the formation of IQ, harman, and norharman by 39%, 67%, and 63%, respectively. In contrast to IQ, the levels of harman and norharman were significantly reduced by the extracts tested. However, no such effects were observed for MeIQx and 4, 8-DiMeIQx. Notably, the inhibitory effect on heterocyclic amines is significantly correlated with the antioxidant potential and total phenolic content of *S. olearleu* extract.

1. Introduction

Considerable attention has been devoted to toxicants produced by cooking in the last decades, particularly in their generation, mitigation, modes of action, and also their influence on human health (Kim et al., 2013). Such toxicants, including polycyclic aromatic hydrocarbons, nitrosamines, and heterocyclic aromatic amines (HAAs), are generated during the cooking of pork and fish. HAAs comprised of two to five condensed aromatic cycles that contain one or more nitrogen atoms. HAAs often have one exocyclic amino group attached to them (Alaejos et al., 2008). They are basically classified into pyrolytic and thermic HAAs, depending on whether their processing temperature is lower or higher than 300 °C. The International Agency for Research on Cancer (IARC) classified eight HAAs, namely MeIQ, 8-MeIQx, 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), AαC, MeAαC, Trp-P-1, Trp-P-2, and Glu-P-1, as possible human carcinogens (class 2B), and 2-amino-3-methyl-3H-imidazo[4,5-F]quinoline (IQ) as a probable human carcinogen (class 2A) (Melo et al., 2008). Health risks may be related to other heat-induced food toxicants. Epidemiological studies indicated that excessive consumption of red pork may increase the risk of certain types of cancer, but more reliable evidences are still scarce. Up until now, there are limited data on the dose-response relationship between HAAs and human cancer. In addition, certain genotypes of HAAs metabolism are also related to cancer risk. Food frequency questionnaires are used to estimate intakes of main HAAs (PhIP and 8-MeIQx) in several foods (Gibis, 2016). Such carcinogenic and mutagenic HAAs can be reduced by many techniques, such as avoiding directly contact of pork surfaces with fire, and adding spices, herbs and fruit extracts during cooking (Rahman et al., 2014). Factors influencing HAAs formation include pork amount and type, storage duration of fresh pork, pH value, cooking method, equipment, temperature, and its duration, and precursor levels in the pork (creatine/creatinine, free amino acids, amino acids, and carbohydrates) (Bhandari, D'arcy, & Young, 2001).

We have been focusing on the investigation of underutilized functional foods, with the hope of finding efficient ways to improve their utilization (Chen et al., 2018; Chen et al., 2016; Teng et al., 2017). *Sonchus oleraceus* Linn is widely consumed as a delicious daily food in China. In South China, especially in the Fujian province, it is a low-

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priced and popular dish for the rural people (Cui et al., 2004; Xia et al., 2011). Several studies have reported that the extracts from *S. oleraceus* (SOE) are beneficial for human physiology with antioxidant activity (Yin et al., 2007), antibacterial activity (Xia et al., 2011), anxiolytic activity (Vilela et al., 2009), and anti-inflammatory activity (Vilela et al., 2010). Phytochemicals isolated from *S. oleraceus* included chlorogenic acid, celeryin, kaempferol, acacetin, isorhamnetin, apigenin 7-*O*-β-D-glucuronatemethyl, apigenin 7-*O*-β-D glucuronide, and luteolin 7-*O*-β-D-glucoside (Yin et al., 2007).

Although *S. oleraceus* is widely used as a flavor improver in pork cooking, its inhibitory effect on HAAs formation during food processing is still not well-understood. In the present study, the inhibitory effects of SOE on HAAs formation in fried pork patties were investigated. Our results indicated that SOE may be used as a natural food additive for reducing HAAs in pork products.

2. Materials and methods

2.1. Reagent

IQ, 4,8-DiMeIQx, MeIQx, PhIP, 9H-pyrido-[4,3-b]indole (Norharman), and 1-methyl-9H-pyrido-[4,3-b] indole (harman) were obtained from ChemCruz (Santa Cruz, CA, USA). Ethyl acetate, acetonitrile, sodium hydroxide, methanol, and petroleum ether were purchased from HuShi (Shanghai, China). Diatomite was purchased from Solarbio (BJ, China). Dichloromethane, boric acid, copper sulphate, and potassium sulphate were purchased from HengXing Chemical (Tianjin, China). Sulfuric acid and hydrochloric acid were purchased from LanXi XuRi Chemical (Tianjin, China). Ammonium acetate, acetic acid, and ammonia were purchased from XiLong science (GD, China). Among them, methanol and acetonitrile were of HPLC grade. All other chemicals were of analytical grade. Ultra-pure water was used throughout the experiment. Bond Elut C_{18} cartridges (3 mL/500 mg) and Bond Elut PRS cartridges (3 mL/500 mg) were ordered from Agilent Technologies (California, USA).

2.2. Raw materials

The whole plant of *S. oleraceus* was obtained from a local supermarket of Fuzhou (China). The samples were pre-blanched, dried, grind and pass through 20 mesh sieve before putting them into the refrigerator (−18 °C, as reported in the label). The powder of *S. oleraceus* was added to the pork puree at 0%, 0.5%, 1.5%, and 3%, respectively.

2.3. Frying conditions

Pan-frying method using a Teflon-coated pan was employed for sample cooking. Two temperatures levels (200 and 230 °C) were utilized and proper cooking time lags was pre-determined according to our preliminary experiments. After that, the samples were chilled naturally under room temperature, then weighed, homogenized makes it a mixture (Tefal, Sarcelles, France), and kept under −70 until use. Surface temperature of the sample was monitored with a Testo 905-T2, thermometer (Lenzkirch, Germany) and the sample was turned at 5-min interval during the cooking process. No salt, spices, fat, or oil was added into the sample.

2.4. Determination of weight loss, lipid, protein, moisture content, pH value

The proximate compositions of the samples, including moisture content, lipid, and protein were determined according to AOAC methods (Horwitz, 2000). The samples were weighted before and 1 h after frying for weight loss calculations. The protein content was analyzed by the Kjeldahl method and the lipid content was determined using the Soxhlet method. Sample pH was detected with a pH meter (Hanna, Vohringen, Germany) after calibration. All experiments were conducted in triplicates.

2.5. Total phenolic content (TPC)

Folin-Ciocalteu method was used for the TPC analysis of the sample (Chen et al., 2016) and the results were expressed as mg gallic acid equivalent per g dry weight (mg GAE/g DW).

2.6. Total flavonoid content (TFC)

TFC analyses were preformed according to a previous study (Chen and Kang, 2014) and the results were expressed as mg rutin equivalent per g dry weight (mg RE/g DW).

2.7. Antioxidant assays

Multiple antioxidant assays including ABTS and DPPH scavenging assays as well as FRAP assay were employed for an overall evaluation of the total antioxidant capacity of the sample (Chen and Kang, 2013). The appropriate quantity of ABTS solution and potassium persulfate was transferred to a 10.00-mL volumetric flask and diluted so that the final concentrations were equal to 7.00 mM and 2.45 mM, respectively. The solution was left in the dark for 12–16 h for the formation of the ABTS radical. Then, the solution of ABTS radical was diluted with ethanol until the absorbance of the solution was equal to 1.0. The radical scavenging activity against ABTS was determined after mixing 0.500 mL sample diluted in 1-butanol with 2.00 mL ABTS solution and measuring the reduction of the absorbance at 734 nm after 15 min incubation. On the other hand, DPPH reagent was prepared by mixing 0.0039 g DPPH powder to 100 μL pure ethanol and equilibrium for 2 h. The extract (100 μL) was then mixed with 900 μL DPPH (100 μM) solution. The sample was shaken vigorously and kept in the dark at room temperature for 30 min. Control was prepared using pure ethanol. The absorbance was measured at 517 nm. The ABTS and DPPH radical scavenging activities were expressed as an inhibition ratio (%) of the sample at 125 μg/mL, and the FRAP activity was expressed as mM of Fe^{2+}/100 g.

2.8. Extraction of HAAs

The ground pork sample of 5 g was dissolved in 30 mL of 1 M NaOH and dispersed evenly for 1 min. The mix was then added with 15 g of diatomite and 40 mL of ethyl acetate. After that, the suspension was homogenized for 30 min using an ultrasound bath. The extraction process was repeated twice before the sample was loaded into the PRS and C_{18} cartridges for HAAs separation. The PRS cartridges (500 mg/3 mL) were preconditioned with 5 mL of dichloromethane and velocity was controlled at 1 mL/min. After the sample supernatant was naturally passed through the PRS cartridge, it was then eluted with 6 mL of 0.01 M hydrochloric acid, followed by 15 mL of methanol-0.1M hydrochloric acid (3/2, v/v) and 2 mL of water. HAAs-containing fraction eluted from the PRS cartridge was loaded in C_{18} cartridges (500 mg/3 mL), which had been preconditioned with a mixture of 5 mL water and 5 mL methanol. This fraction of amines was eluted with 2 mL of mixture (methanol: 25% aqueous ammonia (9:1, v/v). The adsorbed polar HAAs were eluted with 20 mL of 0.5 M ammonium acetate (pH 8.0). C_{18} cartridges were then washed with ultrapure water (2 mL) and dried, and the polar fraction of amines was recovered with 2 mL of the methanol and ammonia mixture. Dried samples were dissolved in 0.3 mL of methanol and filtered through 0.22 μM filters prior to HPLC analysis.

2.9. HAAs analysis

HPLC with a fluorescence detector was employed for HAAs analysis. Isolation was performed on a Tosoh ODS HYPERSIL column at 40 °C

(250 × 4.0 mm, Stuttgart, Germany) with a mobile phase consisting of solvent A: methanol/acetonitrile/water/acetic acid (8/14/76/2, v/v/v/v), adjusted to pH 5.0 with 25% ammonium hydroxide, and solvent B of acetonitrile. The gradient program was as follows: 0% B, 0–12 min; 0–30% B, 12–20 min; and 30% B, 20–35 min. The flow rate was 1.0 mL/min. The injection volume was 10 μL. Recovery rates for HAAs were evaluated by spiking certain concentrations of HAAs (0.1, 0.5, 1.0, 2.5, and 5.0 ng/g) into the raw sample (Chen et al., 2016). All experiments were performed in duplicate. The identification of HAAs was confirmed by comparing the retention time with standard compound. Linear regression (concentration of compound against peak area) equation was calculated for individual HAAs in mix stock solutions. Concentrations of HAAs in the samples were calculated using the linear regression equations.

2.10. Statistical analysis

All analyzes were conducted in triplicate and statistical analyzes were performed using the Statistical Package for DPS 16.05 system (Zhejiang University, Hangzhou, China). The analysis of variance was performed at the 5% level of significance.

3. Results and discussion

3.1. wt loss, lipid, protein, moisture content, pH value

The changes of proximate compositions, such as weight loss, lipid, moisture, and pH values for pork samples cooked with or without SOE are shown in Table 1. The weight loss, pH, moisture, protein, and lipid contents in pork without SOE were 41.54%, 7.31, 0.58%, 37.54% and 0.3%, respectively, when heating temperature was settled at 200 °C for 45 min. A significant difference was observed when the cooking temperature was increased to 230 °C for 105 min, under which the weight loss, pH, moisture, protein, and lipid contents of the sample were 60.35%, 6.31, 0.28%, 54.78% and 0.46%, respectively. Similar data for pork have been published in the literature (Cofrades et al., 2000).

As shown in Fig. 1, a higher temperature cooking process evaporates water and reduces the moisture content of the pork. Accompanying the loss of moisture, the protein content of the pork improved. Both of cooking temperatures and methods significantly affect the variation of the protein and moisture contents ($p < 0.05$). Cooking at 230 °C for 105 min consistently reduced moisture content and increased protein content of the pork patties, regardless of the amount of SOL incorporated in the patties. The weight loss and protein were supposed to increase proportionally after two ways of cooking, due to the lowered moisture content. The lipid content of pork patties didn't significantly increase after cooking. Besides, over-cooked group (230 °C, 105 min) had declined pH values than the normal group (200 °C, 45 min). SOE had no effects on the pH value or proximate compositions of the pork samples as compared to the controls.

Weight loss during the cooking process is primarily associated with the way of cooking, particularly the cooking temperature and duration.

Fig. 1. Photographs of grilled patties containing different amounts of SOE.

Using the frying-cooking method led to a great decrease of weight loss, elevated cooking temperature and cooking time very significantly improved the weight loss during the process ($p < 0.01$). Notably, the weight loss of the sample is not only closely associated with water loss, but also led to declines in proteins during the cooking process (Aaslyng et al., 2003; Tornberg, 2005).

3.2. Antioxidant capacity, TFC, and TPC in SOE

The effects of pan-frying on TFC and TPC in SOE are shown in Table 2. Raw unheated SOE contained highest TFC and TPC. The amount of TPC determined in the raw extract (8.18 mg/g dry weight) was 4 times higher than the results reported by Ninfali and Bacchiocca (2003) (8.2 mg/100 g of fresh weight). It may be explained as the discrepancies in species, soil compositions, cultivation location, and climate, and even the application of a less accurate method of analysis, such as TEAC assay affects (Ninfali and Bacchiocca, 2003). It should be noted that the quantification of TFC showed an approximately 2.72 times higher than that of TPC. The TFC ranged from 3.64 to 41.49 mg RE/g DW as shown in Table 2. The lowest TFC of 3.64 mg RE/g DW was detected after 60 °C pan-frying, whereas the highest TFC level (41.49 mg RE/g DW) was found for the highest temperature of 230 °C tested in the study.

As shown in Table 2, the DPPH radical scavenging activities of SOE ranged from 16.54 to 65.02%, and significant differences were observed when the cooking temperature was elevated. Lower DPPH radical scavenging potential of SOE is corresponding with lower TPC and TFC in the sample (Chen et al., 2012). The results from ABTS and FRAP assays were correlated well with each other. The highest values for FRAP (1.63 mM of Fe^{2+}/100 g) and ABTS inhibition (67.93%) were detected in the SOE after 230 °C heating, whereas the lowest antioxidant activity was detected in 60 °C heating ($p < 0.05$).

Generally, thermal processing of SOE with elevated temperatures (200 and 230 °C) increased its TPC and TFC as well as its antioxidant potential. However, significant losses of TPC (35%) and TFC (84%) were found under the 60 °C treatment. The thermal processing was negatively related to either phenolic or flavonoid contents in foods, such as frozen broccoli (Ozdal et al., 2013; Bunea et al., 2008). By contrast, the present work now offered evidence that TPC and TFC

Table 1
wt loss, pH values, moisture content, lipid content, and protein content of pork sample under cooking process.

S. olearleu	Treatment	Weight Loss	pH	Moisture (g/g)	Protein (g/100 g)	Lipid (g/g)
0%	200 °C, 45 min	41.54 ± 1.12	7.31 ± 0.64	0.58 ± 0.02	37.54 ± 0.99	0.30 ± 0.04
	230 °C, 105 min	60.35 ± 3.26	6.31 ± 0.05	0.28 ± 0.04	54.78 ± 0.12	0.46 ± 0.03
0.5%	200 °C, 45 min	41.63 ± 2.23	7.26 ± 0.14	0.45 ± 0.05	37.58 ± 1.55	0.31 ± 0.02
	230 °C, 105 min	63.34 ± 2.62	6.18 ± 0.03	0.23 ± 0.11	60.16 ± 1.42	0.36 ± 0.01
1.5%	200 °C, 45 min	41.41 ± 2.17	7.70 ± 0.09	0.55 ± 0.02	36.62 ± 0.56	0.38 ± 0.05
	230 °C, 105 min	63.65 ± 2.01	6.16 ± 0.04	0.21 ± 0.03	59.41 ± 1.98	0.33 ± 0.04
3.0%	200 °C, 45 min	40.33 ± 0.03	6.97 ± 0.73	0.52 ± 0.01	35.79 ± 1.11	0.38 ± 0.01
	230 °C, 105 min	60.37 ± 3.49	6.12 ± 0.12	0.18 ± 0.07	55.26 ± 1.42	0.37 ± 0.01

Data are expressed as means of triplicate experiments and were shown as mean ± SD.

Table 2
TPC, TFC and antioxidant activity of SOE during different heat treatment.

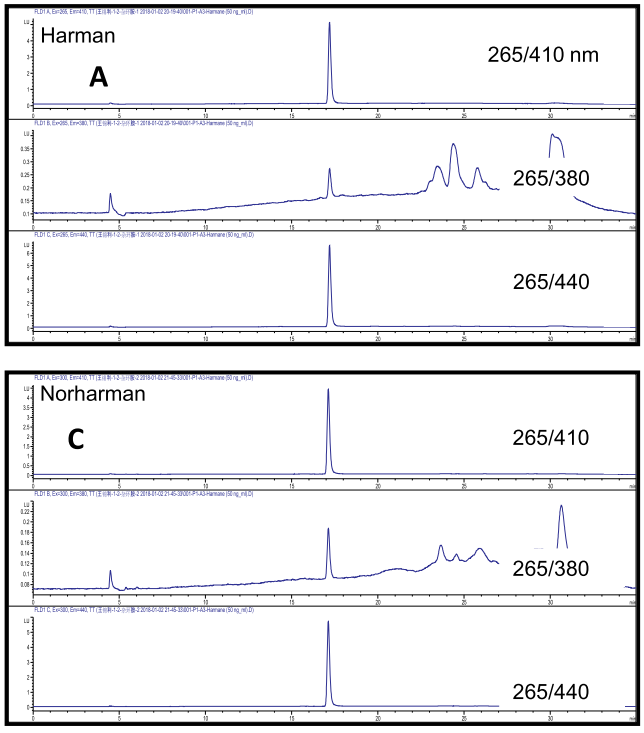
Treatment	TPC mg GAE/g D.W	TFC RE/g D.W.	DPPH %	FRAP mM of Fe^{2+}/100g	ABTS %
RAW	8.18 ± 1.12b	22.32 ± 0.68c	48.85 ± 1.70b	1.10 ± 0.01b	68.85 ± 2.10a
60 °C	5.30 ± 0.41c	3.64 ± 2.21e	16.54 ± 1.72d	0.47 ± 0.02d	37.38 ± 4.65e
100 °C	9.13 ± 0.49b	17.04 ± 0.58d	40.48 ± 1.20c	0.87 ± 0.03c	49.43 ± 1.12d
200 °C	9.64 ± 0.57b	34.59 ± 0.44b	61.74 ± 6.14a	1.29 ± 0.07 ab	53.45 ± 6.20c
230 °C	10.14 ± 0.29a	41.49 ± 1.98a	65.02 ± 6.70a	1.6 ± 0.06a	67.93 ± 1.86b

a Antioxidant activity analyzed by DPPH and ABTS scavenging at the concentration of 125 μg/mL. FRAP was expressed as mM of Fe^{2+}/100g). TPC (total phenolic content) expressed as gallic acid of applied dry weight (mg GAE/g D.W) and TFC (total flavonoid content) as mg RE/g D.W.
Means in rows for each vegetable followed by different letters differed significantly ($p < 0.05$).

increased with elevated temperature (from 100 to 230 °C). It may contribute to the blanching step and further thermal process, during which vegetable tissues become softened through the breakdown of lignocellulosic structure, depolymerisation of the lignin components and defibration (Mazzeo et al., 2011), causing complex changes on chemical compositions of SOE. 0 to100 °C may not reach the temperature at which the lignocellulosic structure was decomposed. Consequently, the phenolic compounds are released easily from food matrix, and extracted into ethanol after cooking, getting higher values in quantity.

3.3. Modification of the analysis method

The HPLC-FLD determination of HAAs showed a good repeatability in different concentrations, as shown in Fig. 2. Correlation coefficients for all HAAs standard curves were more than 0.999, indicating a good linearity and accuracy. An external calibration quantification method was well-established and deemed suitable for further experiment. The detection limits for fluorescence based on a signal-to-noise ratio were higher than those for UV, since the sensitivity of fluorescence detection is higher. Relatively low limit of detection by UV detector for IQ, MeIQ, MeI-Qx and 4,8-DiMeIQx were recorded (data not shown) and the results were compared with Gerbl et al. (2004). Fig. 2A and C shows a typical chromatogram of the harman standard monitored at 265 nm (excitation wavelength) by fluorescence detector. Separation was completed at around 17 min, which was quite similar to that reported by Herraiz (2000). Fig. 2B and D shows the chromatograms of the harman and norharman standards monitored at 300 nm. Under our experimental conditions, no interfering peak was eluted in the region of interest (excitation wavelength of 265 nm and absorption wavelength



E

Sample	Ab (nm)	Rt (min)	Peak height	Peak area
Harmane	265/410	17.198	5.0	54.3
	265/440		6.5	70.5
Norharmane	265/410	16.148	4.1	45.5
	265/440		3.9	43.3

F

Sample	Ab (nm)	Rt (min)	Peak height	Peak area
Harmane	300/410	17.126	4.4	47.8
	300/440		5.7	62.1
Norharmane	300/410	16.197	3.3	36.1
	300/440		3.1	34.3

Fig. 2. The chromatograms obtained by using HPLC-FLD for harman and horharman standard under optimum conditions.

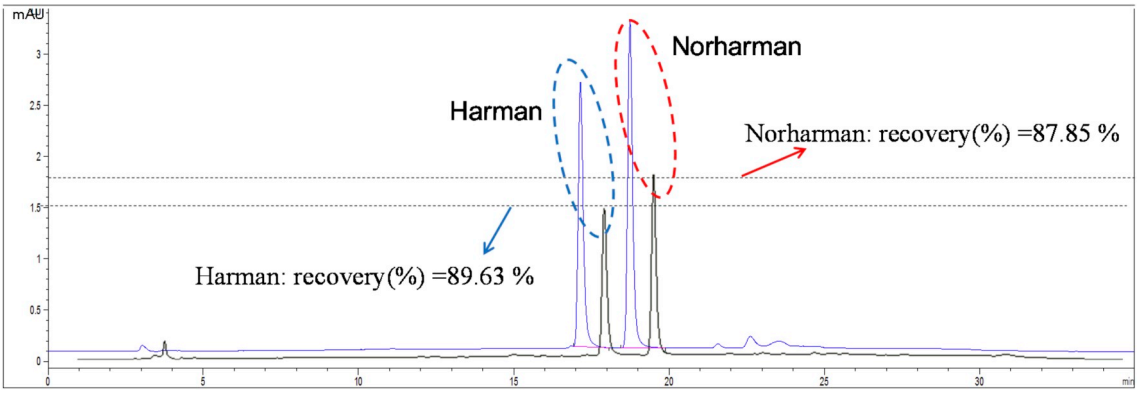


Fig. 3. The recoveries (%) of HAAs analyzed.

of 410 nm). Therefore, as shown in Fig. 2E and F, the quantification was based on the comparison of the analytic peak areas versus an externally generated calibration.

3.4. Recoveries

Recoveries of norharman and harman are compared with related literature for HAAs (Liao et al., 2010; Gibis and Weiss, 2012). The HPLC chromatograms of harman (50 ng/g) and norharman (50 ng/g) mixed stock solutions are shown in Fig. 3. Recoveries of harman and norharman were higher than those reported by Gross and Grüter (1992) (33–85%).

3.5. Effect of processing variables on HAAs content

Table 3 showed the effects of processing variables on the HAAs contents of the cooked patties as quantified by HPLC analysis and expressed in ng/g cooked patty. The HAAs detected in the control pork patty after fried at 200 °C for 45 min were IQ (6.51 ± 0.76 ng/g), norharman (0.45 ± 0.02 ng/g) and harman (0.30 ± 0.09 ng/g) (Table 1). The average levels of HAAs in the control samples cooked at 230 °C for 105 min were determined to be 8.5 ng/g (IQ), 0.65 ng/g (norharman), 0.4 ng/g (harman), and 8.32 ng/g (PhIP). Kondjoyan et al. (2014) applied an air-roasting cylinder for pork cooking and concluded that less HAAs was formed in the colored crusts on the surface of pork pieces than in pork slices under the same heating condition. This can be explained as the result of the temperature of the whole slice increasing faster than the crust. It also can be observed that creatine level decreased in pork slices, resulting in increased PhIP and IQx yields. Thus, these specific heating conditions reduce its HAAs content. Similar results were found by Bordas et al. (2004) who reported that the harman, norharman, and PhIP contents ranged from 3.5 to 10.7 ng/g in pork balls fried at 175 °C for 60 min, whereas lower levels were observed for beef cooked under the same condition. Cheng et al. (2007) reported that the extracts of apple, elderberry, grape seed, and pineapple inhibited the formations of MeIQx, 4, 8-DiMeIQx, and PhIp in pork patties. These findings are in agreement with several previous studies (Wong et al., 2012; Szterk, 2015; Shabbir et al., 2015). However, MeIQx and 4, 8-DiMeIQx were not detected in our samples even at the highest cooking temperature of 230 °C. And the highest IQ and PhIP levels were observed in the control pork samples cooked at 230 °C, but their production amount were remarkably decreased after the addition of 3.0% SOE. These results were consistent with information reported by Chen et al. (1992) who found that food additives including ascorbic acid, bisulfide, nitrite, vitamin E and liquid smoke could prevent the formation of HAA in cooked pork samples during frying, and showed that these reducing agents were more effective than chelating agents. Tsen, Ameri, and Smith (2006) demonstrated that different concentrations of rosemary extract had no effect on the levels of norharman and harman in fried beef patties, but MeIQx and PhIP formations were inhibited by the extracts. By adding antioxidant spices such as onion and garlic extracts to marinades prior to cooking, HAAs formation can be effectively reduced (Shin et al., 2002). Similarly, Keşkekoğlu and Üren (2014) have shown that 0.5% pomegranate seed extract inhibited the formations of PhIP, norharman, IQ, and MeIQx in beef balls (Keşkekoğlu and Üren, 2014). Moreover, total HAAs formation was decreased by 39% and 46% in beef balls cooked by charcoal-barbecue and deep-fat frying, respectively. In the chicken balls, the highest inhibitory effects were 75% for PhIP, 57% for norharman, 28% for harman, 46% for IQ, and 49% for MeIQx. The total HAAs yielded in the chicken balls was increased by 70% under oven roasting, whereas it can be lowered by 49% when the pomegranate seed extract was added during deep-fat frying cooking.

4. Conclusion

This study demonstrated that SOE can effectively inhibit the generation of HAAs, specifically IQ, harman, norharman, and PhIp, in port patties during high-temperature cooking. HAAs levels were significantly reduced with increase in the amount of SOE incorporated into the pork patties. However, in this study, no remarkable effect of *S. olearleu* extract was observed to reduce neither MeIQx nor 4,8-DiMeIQx

Table 3
HAAs in pork samples, supplemented with different amounts of SOE.

Treatment	SOE %	IQ ng/g	4,8-DiMeIQx ng/g	Norharmane ng/g	Harmane ng/g	PhIP ng/g
200 °C 45 min	0.0	6.51 ± 0.76	ND	0.45 ± 0.02	0.30 ± 0.09	ND
	0.5	5.60 ± 1.62	ND	0.14 ± 0.01	0.04 ± 0.02	ND
	1.5	5.56 ± 3.91	ND	0.19 ± 0.02	0.06 ± 0.01	ND
	3.0	4.85 ± 0.92	ND	0.37 ± 0.02	0.11 ± 0.02	ND
230 °C 105 min	0.0	8.50 ± 1.82	ND	0.65 ± 0.01	0.40 ± 0.01	8.32 ± 1.77
	0.5	5.18 ± 0.37	ND	0.23 ± 0.30	0.13 ± 0.01	8.57 ± 0.94
	1.5	3.91 ± 0.66	ND	0.26 ± 0.05	0.30 ± 0.02	5.90 ± 2.70
	3.0	4.28 ± 0.13	ND	0.45 ± 0.01	0.28 ± 0.04	7.48 ± 2.54

content, which might have been due to differences in pork kinds and its recipes preparation, and cooking procedures including cooking temperatures, times and turning frequency of pork. Pan-fried SOE with elevated temperatures (200 °C and 230 °C) increased its TPC and TFC contents as well as its antioxidant activity. Conversely, lower temperatures (60 °C and 100 °C) will reduce its TPC, TFC contents and antioxidant activity. The possible reason was the structural decomposition of lignocellulose in elevated temperature environment (200 °C and 230 °C), phenolic compounds are more easily released, and in lower temperature environment (60 °C and 100 °C), the temperature at which the lignocellulosic structure decomposes is not reached. A strong linear correlation between IQ, harman, norharman or PhIP contents and the gallic acid equivalents phenol content from SOE was found in our study. Similarly, the total flavonoid content was correlated with the detectable HAA contents, our correlation analysis also suggest that the inhibition of IQ, harman, norharman and PhIP generation in cooked pork patties was linked to the antioxidant capacity and TFC of SOE.

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