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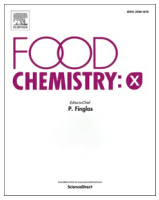

Inhibitory effects of longan seed extract on polycyclic aromatic hydrocarbons formation and muscle oxidation in baked mutton kebabs

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ABSTRACT

Longan seeds, rich in phenolic compounds with antioxidant properties, are an underestimated by-product of longan processing. Polycyclic aromatic hydrocarbons (PAHs), which are carcinogenic and mutagenic, are produced during the cooking of meat products at high temperatures. The effects of different concentrations of longan seed extract (LSE, 0.2, 0.6, 1.0 mg/mL) on the formation of PAHs and muscle oxidation in mutton kebabs were investigated. Mutton kebabs were baked at 150, 200, 250 °C for 20 min, respectively, and the contents of PAHs, the degree of lipid and protein oxidation were evaluated. The results showed that LSE exhibited positive effects in inhibiting total PAHs formation (range from 14.9 to 48.8 %), decreasing the thiobarbituric acid reactive substances (TBARS) values (range from 17.1 to 39.1 %), reducing carbonyl content (range from 22.0 to 51.2 %) and increasing sulfhydryl content (range from 18.6 to 51.8 %). This study provided a guidance and potential solution for reducing the content of PAHs and muscle oxidation levels in baked meat.

1. Introduction

Mutton is characterized by the rich content of protein, vitamin B, iron, zinc, selenium, and low-fat level, which occupies an important position in the daily diet of residents. With the improvement of the standard of living of the people, the consumption of mutton products is increasing. However, many harmful compounds such as PAHs, heterocyclic amines and advanced glycation end products are formed during thermal processing of mutton products such as baking, smoking, and frying (Cho et al., 2023; Singh, Agarwal, & Simal-Gandara, 2020; Wen et al., 2022). PAHs are a category of organic compounds consisting of two or more fused aromatic rings. Currently, there are more than 20 kinds of PAHs commonly found in food, among them, 16 kinds of PAHs have been categorized as priority control contaminants by the US Environmental Protection Agency, including naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), chrysene (Chr), pyrene (Pyr), fluoranthene (Flt), benzo (*a*) anthracene (BaA), benzo (*b*) fluoranthene (BbF), benzo (*a*) pyrene (Bap), benzo (*k*) fluoranthene (BkF), benzo (*g, h, i*) perylene (BghiP), indeno(*1, 2, 3-c, d*)pyrene (InP) and dibenzo (*a, h*) anthracene (DahA) (Min, Patra, & Shin, 2018). The studies indicated that PAHs could harm human health and might lead to colorectal, pancreatic, stomach, lung and oesophageal cancers (Hamidi, Hajeb, Selamat, & Abdull Razis, 2016). The precise mechanisms of PAHs formation in food are still unclear. However, it is generally accepted that their formation is due to complex reactions of free radical-mediated pyrolysis and thermosynthesis of organic substances such as fats, proteins and carbohydrates (Min et al., 2018; Singh et al., 2020; Yu et al., 2023). It is well known that the levels of PAHs are influenced by a variety of factors, such as the type of food, processing method, cooking time and temperature, and food additives (Lu, Kuhnle, & Cheng, 2018; Min et al., 2018; Onopiuk, Kołodziejczak, Szpicer, Wojtasik-Kalinowska, Wierzbicka, & Półtorak, 2021; Siddique, Zahoor, Ahmad, Zahid, & Karrar, 2021).

PAHs are produced from precursors through a complex process and their formation involves a series of free radical reactions, however, antioxidants can scavenge free radicals generated during hydrocarbon cleavage and aromatic compound cyclization, thus preventing the formation of PAHs (Lu et al., 2018; Onopiuk et al., 2021). Therefore, the addition of antioxidants to meat products is considered an efficient means to decrease the levels of PAHs in cooked meat products. Antioxidants can be divided into synthetic and natural antioxidants depending on their source. Synthetic antioxidants such as tertiary butylhydroquinone and utylated hydroxytoluene have been banned in some countries as they have carcinogenic potential (Oz & Kaya, 2011).

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Consumers prefer to use natural antioxidants in food processing. Natural antioxidants are widely distributed in plants, animal tissues and microorganisms. Most natural antioxidants are obtained from plants, such as tea, spices, vegetables, fruits and their waste. Currently, plant extracts as natural and safe antioxidants have been widely used to inhibit the formation of PAHs in meat processing. Plant extracts have antioxidant activity and can prevent or scavenge the production of free radicals, thereby reducing the levels of PAHs. Some reports found that plant by-products (grape seeds, bay leaves, coriander root, etc.) were efficient in inhibiting the formation of PAHs in meat products owing to their antioxidant ingredients (Onopiuk et al., 2022; Shen, Huang, Tang, Zhan, & Liu, 2022; Yu et al., 2023).

Longan seeds are approximately 12.5–21.1 % of the whole fruit's fresh weight, and they are generally discarded or burned as fuel after processing, resulting in a serious waste of resources and environmental pollution (Rakariyatham, Zhou, Rakariyatham, & Shahidi, 2020). Studies have suggested that the seeds of longan possess abundant phenolic and flavonoid compounds, which have powerful antioxidant activity (Tang et al., 2019). Most studies have concentrated on the medicinal value of longan seeds, such as hypoglycemic, anti-glycation, anti-cancer, and memory enhancement (Zhu, Wang, Sun, Yang, Duan, & Jiang, 2019). Nevertheless, the information on the utilization of longan seeds in food is limited. So far, LSE has been reported in the literature for the preparation of packaging films to prolong the shelf life of food, but its inhibitory effect on PAHs formation and muscle oxidation in baked meat has not been reported. Therefore, this study intended to enquire into the effects of different concentrations of LSE on the levels of PAHs and muscle oxidation in mutton kebabs, to provide a theoretical foundation for the control of PAHs formation and muscle oxidation in meat products.

2. Materials and methods

2.1. Materials and reagents

Longan (cultivar Chuliang) and mutton were bought from Supermarket (Lanzhou, China). The 16 PAHs standards (Nap, Acy, Ace, Flu, Phe, Ant, Chr, Pyr, Flt, BaA, BbF, BaP, BkF, BghiP, InP and DahA) were purchased from Changzhou Tan-Mo Technology Co., Ltd. (Changzhou, China). Acetonitrile, hexane, ethanol and dichloromethane were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China). Folin-Ciocalteu reagent, Rutin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Gallic acid, 5, 5-dithiobis-(2-nitrobenzoicacid) (DTNB), and 2-thiobarbituric acid (TBA) were purchased from Shanghai yuan ye Bio-technology Co., Ltd. (Shanghai, China). 2,4-dinitrophenylhydrazine (DNPH) was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China).

2.2. Preparation of LSE

The washed longan seeds were dried at 50 °C for 24 h and then ground into powder using a grinder (XM-2500, Zhejiang, China), and passed through a 60-mesh screen (screen size of 250 mm). The longan seed powder was placed in ethanol extraction solvent [60 % ethanol (v/v)] with a solid–liquid ratio of 1:25 (g/mL), followed by treatment with ultrasonic equipment (KQ-500E, Kunshan, China) at 500 w, 70 °C for 1 h. After that, the extracts were vacuum filtered by a vacuum pump (SHB-III, Changsha, China) and medium-speed qualitative filter paper (102, Liaoning, China), and the filtrate was collected. The filtrates were concentrated by rotary evaporator (RE52CS, Shanghai, China) at 60 °C. The concentrates were subsequently freeze-dried by a freeze-dryer (FC-18A, Hebei, China) and preserved in the refrigerator (BD/BC-408B, Zhejiang, China) at − 20 °C.

2.3. Total phenolic content (TPC) assay for LSE

The Folin-Ciocalteu colorimetric approach was performed to assess the TPC of LSE (Walter, Marchesan, Sachet Massoni, da Silva, Sarzi Sartori, & Ferreira, 2013). Firstly, 0.1 mg/mL of gallic acid standard solution was made. Then different volumes of gallic acid standard solutions (0, 0.1, 0.2, 0.3, 0.4, 0.5 mL) were mixed with Folin-Ciocalteu reagent (1 mL, 1moL/L) and 1 mL of 10 % Na_2CO_3 solution, respectively, which reacted for 1 h at room temperature. The absorbance values of the standard solutions were tested at 760 nm using a spectrophotometer (Shimadzu model UV-1601, Shizuoka, Japan). Among them, "0 mL" was the control group. The standard curve was obtained ($y = 11.22x\text{-}0.0018$, $R^2 = 0.9998$) (see Supplementary Fig. S1). The TPC in LSE was counted using the standard curve.

2.4. Total flavonoid content (TFC) assay for LSE

The TFC of LSE was assessed through the approach of Kim, Chun, Kim, Moon, and Lee (2003). At first, 0.2 mg/mL of rutin standard solution was prepared with ethanol solvent [80 % ethanol (v/v)]. Then different volumes of rutin standard solutions (0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5 mL) were mixed with 0.3 mL of 5 % Na_2NO_2, 0.3 mL of 10 % $Al(NO_3)_3$ and 4 mL of 4 % NaOH solution, and the volume of the mixture was fixed to 10 mL with ethanol solvent [80 % ethanol (v/v)], and the reaction was carried out for 15 min. The absorbance value of the standard solution was tested by spectrophotometer at 510 nm. Among them, "0 mL" was the control group. The standard curve was obtained ($y = 0.2363x\text{-}0.0022$, $R^2 = 0.9997$) (see Supplementary Fig. S1). The TFC in LSE was counted using the standard curve.

2.5. DPPH assay for LSE

The scavenging ability of LSE on DPPH radicals was assessed through the means by Wang et al. (2018) with slight corrections. The DPPH solution of 0.1 mg/mL was prepared with ethanol solvent [95 % ethanol (v/v)]. LSE was dissolved in distilled water making concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL. 0.2, 0.4, 0.6, 0.8, 1.0 mg/L of LSE - water solutions were marked as LSE0.2, LSE0.6, LSE0.8 and LSE1.0, respectively. LSE0.2 (2.5 mL), LSE0.4 (2.5 mL), LSE0.6 (2.5 mL), LSE0.8 (2.5 mL), LSE1.0 (2.5 mL) and 2.5 mL of 0.1 mg/mL DPPH solution were mixed separately and reacted for 30 min away from light, and anhydrous ethanol was the blank control. The absorbance value was measured at 517 nm using a spectrophotometer and recorded as A_1. The DPPH solution was replaced with an anhydrous ethanol solution using the above method and the absorbance value was recorded A_2. The DPPH solution (2.5 mL, 0.1 mg /mL) and 2.5 mL anhydrous ethanol solution were mixed and reacted for 30 min away from light, then the absorbance value was recorded A_3. The DPPH radical that scavenged activity was counted through the following formula.

$$DPPH\ radical\ scavenging\ activity(\%) = \left(1 - \frac{A_1 - A_2}{A_3}\right) \times 100$$

2.6. Preparation of mutton kebabs

The mutton was chopped into small pieces of 1.5 × 1.5 × 1.5 cm^3 after removing visible fat and connective tissue. LSE was dissolved in distilled water making concentrations of 0.2, 0.6 and 1.0 mg/mL. Subsequently, the cut meat pieces were immersed in this solution and stored in a refrigerator (BCD-649WE, Qingdao, China) at 4 ℃. The soaking time was 4 h, and the ratio of meat to the soaking solution was 1:1 (g/mL). Among them, the pieces of mutton soaked in distilled water were used as the control group. After soaking, the pieces of mutton were removed and skewered into skewers. Then mutton kebabs were baked using an oven (HB233ABS1W, Beijing, China) at 150, 200, 250 °C, respectively. The kebabs were baked for 10 min, then turned over and

baked for 10 min. After baking, the mutton kebabs were cooled and then preserved in the refrigerator at −20 °C.

2.7. Extraction and determination of PAHs

The PAHs content was measured through the means by Wang et al. (2018) with slight corrections. In brief, 3 g of chopped meat samples was placed into a 50 mL centrifuge tube, subsequently, 20 mL of acetonitrile and 10 mL of *n*-hexane saturated acetonitrile solution were added. Samples were homogenized for 30 s by homogenizer (FH-2A, Ningbo, China) and then sonicated at 40 °C for 30 min. The samples were then centrifuged at 2387 × g for 30 min by centrifugal machine (TGL-16MC, Hunan, China) and the acetonitrile layer of the lower part of the tube was collected into a distillation flask. The residual solution was extracted again following the above steps. The extracted solutions were combined and rotary evaporated at 35 °C to near dryness. 6 mL of *n*-hexane was added to the residue, loaded into a molecular imprinting column of PAHs (PAH-MIP, Shanxi, China), activated with 5 mL of dichloromethane, conditioned with 10 mL of *n*-hexane for purification. 9 mL *n*-hexane/dichloromethane (30:70, v/v) was used to elute the PAH-MIP. All eluents were collected in a test tube and evaporated to near dryness under a stream of nitrogen. The concentrate was dissolved in 1 mL of acetonitrile, filtered through the 0.22 μm nylon membrane filter (SCAA-104, Shanghai, China) and then injected into the 1.5 mL chromatography injection vial.

PAHs were separated and quantified using Agilent 1260 InfinityII high-performance liquid chromatography with diode array detection and fluorescence detection (HPLC-DAD/FLD) following the means of Wang et al. (2018) with slight corrections. Separation of PAHs was performed with an Agilent SB C18 column (5 μm, 4.6 mm × 250 mm). The mobile phases were water (A) and acetonitrile (B), respectively. After injection of 5 μL of the sample, the gradient elution program was 5 min, 27 % A and 73 % B; 25 min, 25 % A and 75 % B; 33 min, 5 % A and 95 % B; 40 min, 0 % A and 100 % B; 45 min, 50 % A and 50 % B. The column temperature was 30 °C and the flow rate was 0.8 mL/min. Acy was measured with a diode array detector (DAD) at 230 nm. The excitation and emission wavelengths were 270 and 324 nm for Nap, Flu and Ace; 248 and 375 nm for Phe and Ant; 280 and 462 nm for Flt; 270 and 385 nm for Pyr, Chr and BaA; 292 and 410 nm for BbF, BkF, Bap and DahA; 274 and 507 nm for InP; 292 and 410 nm for BghiP. The substances were identified by comparing the retention times of the samples and the reference standard mixture of PAHs. Standard curves were listed in Table 1 for the quantification of each PAH compound.

2.8. TBARS

The TBARS values of mutton kebabs were tested through the means of Kilic and Richards (2010) with some modifications. Above all, the 2.5 g of chopped meat samples was placed into a 50 mL centrifuge tube, subsequently, 25 mL of trichloroacetic acid solution (7.5 % trichloroacetic acid, 0.1 % ethylene diamine tetraacetic acid) were added and the mixture was blended by vortex mixer (QL-866, Haimen China). Then, the mixture was placed on a constant temperature shaker (THZ-82N, Shanghai, China) at 50 °C for 30 min. It was taken out from the constant temperature shaker and cooled, followed by filtering through medium-speed qualitative filter paper and collecting the filtrate. After that, aspirated 5 mL of the above filtrate, another 5 mL of trichloroacetic acid solution was the sample blank, and added 5 mL of 0.02 mol/L TBA solution to them respectively, placed in a water bath at 90℃ for 30 min, removed and cooled. TBARS values were counted through the standard curve of 1,1,3,3-tetraethoxypropane. The malondialdehyde (MDA) content (mg/kg) was used to express TBARS values.

2.9. Carbonyl and sulfhydryl content

The myofibrillar protein (MP) of baked mutton kebabs was extracted using methodology documented by Park, Xiong, and Alderton (2007). Added 40 mL of standard salt solution (20 mmol/L potassium phosphate buffer, 0.1 mol/L KCl, 2 mmol/L $MgCl_2$, 1 mmol/L Ethylene glycol bis (2-aminoethylether)-N, N, N', N'-tetraacetic acid, pH 6.8) to 4 g of meat sample. The mixture was homogenized (13000 rpm, 10 s) and then centrifuged at 1000 × g for 10 min at 4 °C, followed by discarding the supernatant. Added 32 mL of 0.1 mol/L KCl solution to the precipitate and the mixture was centrifuged at 1000 xg for 10 min at 4 °C, then the supernatant was discarded. The precipitate was dissolved in 4 mL of 0.1 mol/L KCl solution and the protein concentration of the extracted MP was calculated using the biuret method.

Carbonyl content of baked mutton kebabs was measured as described by Wang et al. (2020). DNPH was added to 0.5 mL of MP solution (5 mg/mL) to form protein hydrazones. Sample absorbance was measured at 370 nm. Carbonyl content was calculated with the molar extinction coefficient of 22000 M^{-1} cm^{-1}.

The sulfhydryl content of baked mutton kebabs was measured as described by Wang et al. (2020). Added 2 mL of urea-sodium dodecyl sulfate solution (SDS) (8.0 mol/L urea, 30 g/L SDS, 0.1 mol/L sodium phosphate buffer, pH 7.4) and 0.5 mL of 10 mmol/L DTNB solution (pH 7.4) to 0.5 mL of MP solution (2 mg/mL), and the absorbance at 412 nm was read after 15 min of reaction at room temperature. Sulfhydryl content was calculated with the molar extinction coefficient of 13600

Table 1
The chemical abstracts service registry number (CAS RN), retention times (Rt), limit of detection (LOD), limit of quantitation (LOQ), calibration curves and regression coefficients of the 16 determined PAHs.

PAHs	CAS RN	Rt(min)	Calibration curve equation	Regression coefficient	LOD (μg/kg)	LOQ (μg/kg)
Nap	91–20-3	10.877	y = 0.1817x-0.0539	R^2 = 0.9998	3.30	10.0
Acy	208–96-8	11.875	y = 0.0906x + 0.9185	R^2 = 0.9937	1.50	4.5
Flu	86–73-7	14.049	y = 1.6177x-1.5811	R^2 = 0.9942	0.65	2.0
Ace	83–32-9	14.385	y = 0.3237x-0.1479	R^2 = 0.9998	0.65	2.0
Phe	85–01-8	15.176	y = 0.3959x-0.2849	R^2 = 0.9974	2.00	6.0
Ant	120–12-7	15.998	y = 0.2402x-0.0317	R^2 = 0.9999	0.33	1.0
Flt	206–44-0	18.229	y = 0.1989x-0.0594	R^2 = 0.9998	0.50	1.5
Pyr	129–00-0	19.575	y = 1.8049x-0.2711	R^2 = 0.9999	0.65	2.0
Chr	218–01-9	23.410	y = 1.9079x-0.5004	R^2 = 0.9999	0.33	1.0
BaA	56–55-3	23.989	y = 1.1874x + 0.0616	R^2 = 0.9994	0.33	1.5
BbF	205–99-2	29.972	y = 0.4078x-0.4268	R^2 = 0.9988	0.33	2.0
BkF	207–08-9	30.773	y = 1.8041x-0.8456	R^2 = 0.9996	0.33	1.0
BaP	50–32-8	31.940	y = 3.2579x-0.9298	R^2 = 0.9999	0.33	1.0
DahA	53–70-3	34.774	y = 0.2214x-0.132	R^2 = 0.9998	0.33	1.0
InP	193–39-5	36.379	y = 0.1194x-0.1864	R^2 = 0.9967	0.33	1.0
BghiP	191–24-2	36.676	y = 0.6400x-0.4254	R^2 = 0.9993	0.33	1.0

M^{-1} cm^{-1}.

2.10. Statistical analysis

All experiments were replicated three times (n = 3). The results were presented as mean ± standard deviation. Data were analyzed by one-way ANOVA using the IBM SPSS 20.0 software (SPSS Inc, Chicago, IL, USA). Duncan's multiple range test ($P < 0.05$) was performed to compare the significance of the difference among mean values.

3. Results and discussion

3.1. TPC, TFC and antioxidant capacity of LSE

Longan seeds are often discarded as waste from the longan processing procedure, however, they possess abundant phenolic substances such as phenolic acids and flavonoids (Rakariyatham et al., 2020). In this study, the TPC and TFC in longan seeds were 25.98 ± 0.54 mg gallic acid equivalents (GAE)/g dry weight (DW) and 17.58 ± 0.65 mg rutin equivalents (RE)/g DW. There were differences in TPC and TFC of longan seeds under different environments and regions. In the Chinese market, common longan cultivars include Shixia and Linglong from Guangxi, Chuliang from Guangdong, and Gushan No.2 from Fujian. There were huge differences in phenolic contents among the different longan cultivars. Tang et al. (2019) found that the TPC and TFC in longan seeds (cultivar Shixia) were 3.77 ± 0.88 mg GAE/g DW and 1.94 ± 0.01 mg RE/g DW, respectively. Moreover, different extraction methods had a significant effect on the phenolic contents. Li et al. (2014) reported the efficiency of different solvents (distilled water, sodium phosphate buffer, hydrochloric acid and ethanol) in extracting phenolics from longan seeds (cultivar Fenglisui), among them, ethanol was the better extraction solvent with an extraction yield of 48.88 ± 1.52 mg/g. As a result, these could explain the difference between the TPC and TFC values of longan seeds in this study and the results reported above.

DPPH is commonly used to estimate the antioxidant capacity of a substance (Ibrahim, Alqurashi, & Alfaraj, 2022). Fig. 1 demonstrated that DPPH scavenging capacity significantly enhanced with the increase of LSE concentrations. The scavenging range of DPPH radicals by LSE was 76.4–91.3 %, which indicated that LSE had a good scavenging effect on DPPH free radicals. Wang, Chen, Wang, and Shi (2023) discovered that the DPPH scavenging activity of extract of longan seeds increased significantly with concentration. Tang et al. (2019) reported that phenolic in longan seeds exhibited a concentration-dependent effect on DPPH radical scavenging activity. The results were in agreement with the above reports, indicating that LSE had the potential to reduce the formation of PAHs due to its antioxidant capacity to scavenge free radicals.

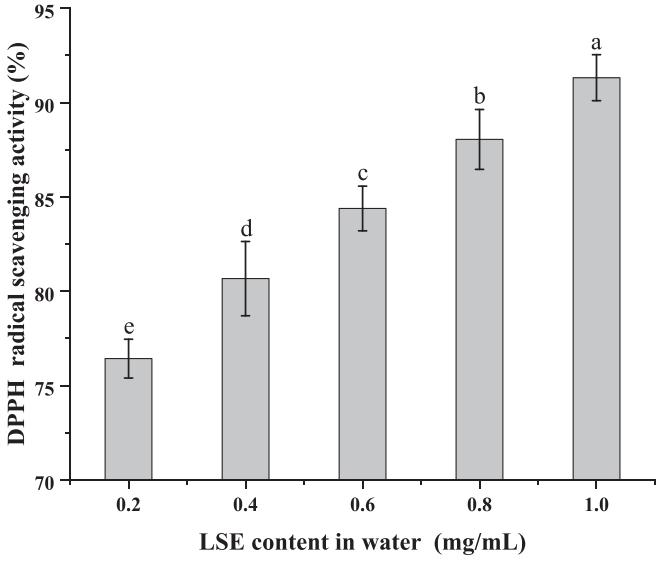

Fig. 1. DPPH radical scavenging activity of LSE - water solution at different concentrations. Data were expressed as mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences among various LSE concentrations ($P < 0.05$).

3.2. Effects of baking temperature and LSE on levels of PAHs in baked mutton kebabs

Table 2 listed the levels of PAHs in baked mutton kebabs at different baking temperatures and concentrations of LSE. The total contents of PAHs in the control group ranged from 87.02 to 178.50 μg/kg, with higher levels of Nap (44.82–62.29 μg/kg), Ace (8.09–37.13 μg/kg), Phe (7.66–17.85 μg/kg) and Ant (14.78–27.13 μg/kg), and the levels of BaA, Chr, BkF and BaP were lower, which were all below 1 μg/kg. In addition, DahA, Inp, Bghip were not detected and were not listed in Table 2. These findings were lower than reported by Cho et al. (2023), who detected total content of 16 PAHs varied from 198.33 to 280.79 μg/kg in charcoal-grilled pork patties. In addition, Flt, Inp was not detected in their samples. Three major reasons could explain these differences. Firstly, this could be because the cooking temperature in this study was lower than theirs (charcoal temperature of 550–600 °C). Secondly, the cooking method was different, they were charcoal grilled while we were oven baked. Finally, the sample they tested was pork, which had a higher fat content than mutton.

By comparing the three groups with different cooking temperatures, at the same concentration of LSE, the contents of total PAHs in the mutton kebab samples increased significantly as the temperature went up ($P < 0.05$). Hui, Fang, Li, and Hamid (2022) found that temperature was a crucial factor affecting the formation of PAHs in meat products. Wen et al. (2022) showed that the levels of PAHs in roast beef increased with roasting temperature (150, 190, 230, 270 and 310 °C). The results were consistent with those reports of the researchers mentioned above. The results suggested that the cooking-induced PAHs content was affected by the heat treatment temperature, which might be due to the fact that high temperatures facilitate the pyrolysis of food components, followed by a series of reactions such as cyclization and polymerization to produce PAHs (Singh et al., 2020). In short, the levels of PAHs in baked mutton kebabs increased with the rising of baking temperature. In addition, all concentrations of LSE reduced the total levels of PAHs in baked mutton kebabs at the three temperatures, with the most effective reductions of 27.3 % (150 °C), 38.0 % (200 °C) and 48.8 % (250 °C), respectively. Obviously, the LSE-treated group was more effective in inhibiting PAHs formation at 250 °C and 200 °C than at 150 °C. Cho et al. (2023) found that 0.4 % ethanolic extract from perilla leaves had a greater inhibitory effect on the formation of PAHs in the well-done pork patties (the internal temperature was about 80℃) in contrast with medium-cooked ones (the internal temperature was about 71℃). Wang, Wang, Li, Xu, and Zhou (2019) discovered that 2 mg/L of protocatechuic acid had a greater inhibitory effect on the formation of PAHs in chicken wings grilled at 240 °C and 270 °C compared to those grilled at 210 °C. In this study, baked mutton kebabs treated with LSE showed stronger inhibition of the content of total PAHs at higher cooking temperatures, which might be attributed to the degradation of hydrolysable tannins in LSE to smaller phenolic compounds by heat treatment, thus improving its antioxidant properties (Zhao, Xi, Zhang, Ma, & Wang, 2021).

In this investigation, different concentrations of LSE treatment at the same baking temperature significantly reduced the contents of total PAHs in contrast with the control group ($P < 0.05$). The LSE0.6 exhibited the strongest inhibition of total PAHs formation in mutton kebabs at the heat treatment temperature of 150 °C, reaching 27.3 %, followed by LSE1.0 (22.5 %), LSE0.2 (14.9 %). The LSE0.6 showed the strongest inhibition of total PAHs formation in mutton kebabs at the heat treatment temperature of 200 °C, reaching 38.0 %, followed by

Table 2
The PAHs contents (μg/kg) of baked mutton kebabs at different concentrations of longan seed extract and baking temperature.

compounds	150℃				200℃				250℃			
	LSE0[A]	LSE0.2[B]	LSE0.6[C]	LSE1.0[D]	LSE0	LSE0.2	LSE0.6	LSE1.0	LSE0	LSE0.2	LSE0.6	LSE1.0
Nap	44.82 ± 1.94^{Ca}	40.27 ± 2.12^{Cb}	36.06 ± 1.60^{Cb}	37.46 ± 2.76^{Cb}	50.36 ± 2.15^{Ba}	45.71 ± 2.72^{Bb}	39.86 ± 1.43^{Bc}	42.23 ± 1.62^{Bbc}	62.29 ± 1.99^{Aa}	51.45 ± 1.57^{Ab}	43.97 ± 1.45^{Ac}	46.99 ± 2.56^{Ac}
Acy	1.42 ± 0.11^{Ca}	1.12 ± 0.07^{Cb}	0.98 ± 0.07^{Cb}	1.04 ± 0.06^{Cb}	1.87 ± 0.11^{Ba}	1.61 ± 0.14^{Bb}	1.25 ± 0.10^{Bc}	1.33 ± 0.11^{Bc}	3.59 ± 0.24^{Aa}	2.52 ± 0.19^{Ab}	1.56 ± 0.16^{Ac}	1.61 ± 0.15^{Ac}
Ace	8.09 ± 0.48^{Ca}	6.14 ± 0.50^{Cb}	5.28 ± 0.33^{Cb}	5.76 ± 0.50^{Cb}	17.38 ± 0.76^{Ba}	11.73 ± 0.54^{Bb}	7.47 ± 0.65^{Bc}	8.62 ± 0.49^{Bc}	37.13 ± 1.56^{Aa}	14.30 ± 0.96^{Ab}	9.86 ± 0.60^{Ac}	11.80 ± 0.88^{Ac}
Flu	5.47 ± 0.38^{Ca}	4.05 ± 0.22^{Cb}	3.56 ± 0.15^{Cb}	3.81 ± 0.28^{Cb}	9.56 ± 0.80^{Ba}	7.35 ± 0.71^{Bb}	5.07 ± 0.37^{Bc}	6.16 ± 0.45^{Bc}	14.86 ± 1.10^{Aa}	9.57 ± 0.68^{Ab}	7.22 ± 0.43^{Ac}	8.11 ± 0.72^{Ac}
Phe	7.66 ± 0.54^{Ca}	6.28 ± 0.53^{Cb}	5.42 ± 0.34^{Cb}	5.93 ± 0.35^{Cb}	12.42 ± 0.86^{Ba}	8.47 ± 0.71^{Bb}	6.58 ± 0.44^{Bc}	7.38 ± 0.59^{Bbc}	17.85 ± 1.08^{Aa}	10.40 ± 0.59^{Ab}	7.77 ± 0.57^{Ac}	8.82 ± 0.67^{Ac}
Ant	14.78 ± 0.80^{Ca}	12.13 ± 0.71^{Cb}	8.34 ± 0.36^{Cc}	9.52 ± 0.65^{Cc}	23.47 ± 0.90^{Ba}	16.17 ± 0.92^{Bb}	10.53 ± 0.77^{Bc}	11.86 ± 1.04^{Bc}	27.13 ± 1.10^{Aa}	18.46 ± 1.17^{Ab}	12.54 ± 0.94^{Ac}	14.28 ± 0.80^{Ac}
Flt	1.98 ± 0.16^{Ca}	1.61 ± 0.07^{Cb}	1.42 ± 0.13^{Cb}	1.53 ± 0.11^{Cb}	3.24 ± 0.21^{Ba}	2.78 ± 0.13^{Bb}	1.94 ± 0.34^{Bc}	1.86 ± 0.17^{Bc}	5.46 ± 0.34^{Aa}	3.54 ± 0.29^{Ab}	2.47 ± 0.15^{Ac}	2.89 ± 0.20^{Ac}
Pyr	2.41 ± 0.18^{Ca}	2.14 ± 0.10^{Cb}	1.95 ± 0.08^{Cb}	2.08 ± 0.15^{Cb}	3.51 ± 0.25^{Ba}	3.10 ± 0.15^{Bb}	2.73 ± 0.13^{Bc}	2.98 ± 0.21^{Bbc}	6.30 ± 0.51^{Aa}	5.58 ± 0.41^{Ab}	3.84 ± 0.29^{Ac}	4.36 ± 0.25^{Ac}
BaA	ND	ND	ND	ND	ND	ND	ND	ND	0.72 ± 0.06^{a}	0.61 ± 0.03^{b}	0.50 ± 0.04^{c}	0.56 ± 0.03^{bc}
Chr	ND	ND	ND	ND	ND	ND	ND	ND	0.47 ± 0.04^{a}	0.49 ± 0.02^{a}	ND	ND
BbF	ND	ND	ND	ND	1.24 ± 0.07^{Ba}	1.06 ± 0.06^{Bb}	0.78 ± 0.06^{Bc}	0.83 ± 0.08^{Bc}	1.72 ± 0.11^{Aa}	1.54 ± 0.11^{Ab}	1.07 ± 0.07^{Ac}	1.22 ± 0.09^{Ac}
BkF	0.22 ± 0.01^{Ca}	0.17 ± 0.01^{Cb}	0.12 ± 0.02^{Cc}	0.14 ± 0.01^{Cc}	0.36 ± 0.03^{Ba}	0.30 ± 0.03^{Bb}	0.23 ± 0.02^{Bc}	0.24 ± 0.03^{Bc}	0.56 ± 0.05^{Aa}	0.44 ± 0.03^{Ab}	0.30 ± 0.02^{Ac}	0.35 ± 0.05^{Ac}
Bap	0.16 ± 0.02^{Ca}	0.13 ± 0.01^{Cb}	0.13 ± 0.01^{Cb}	0.14 ± 0.01^{Cb}	0.31 ± 0.03^{Ba}	0.26 ± 0.02^{Bb}	0.24 ± 0.02^{Bb}	0.24 ± 0.03^{Bb}	0.42 ± 0.03^{Aa}	0.38 ± 0.03^{Ab}	0.30 ± 0.02^{Ac}	0.34 ± 0.02^{Abc}
Total PAHs	87.02 ± 4.06^{Ca}	74.03 ± 3.47^{Cb}	63.26 ± 2.73^{Cc}	67.40 ± 3.79^{Cbc}	123.73 ± 5.44^{Ba}	98.56 ± 4.78^{Bb}	76.66 ± 3.68^{Bc}	83.74 ± 4.41^{Bc}	178.50 ± 7.90^{Aa}	119.28 ± 5.20^{Ab}	91.41 ± 4.22^{Ac}	101.34 ± 5.91^{Ac}

Data were expressed as mean ± standard deviation (n = 3). ND: not detected. Results with different uppercase letters in the same row indicate significant differences between three baking temperature samples with same LSE content treatment at the level $P < 0.05$, and results with different lowercase letters in the same row indicate significant differences among the same baking temperature samples with different LSE content treatment at the level $P < 0.05$.

[A] LSE0: 0 mg/mL longan seed extract - water solution
[B] LSE0.2: 0.2 mg/mL longan seed extract - water solution
[C] LSE0.6: 0.6 mg/mL longan seed extract - water solution
[D] LSE1.0: 1.0 mg/mL longan seed extract - water solution

LSE1.0 (32.3 %), LSE0.2 (20.3 %). The LSE0.6 demonstrated the strongest inhibition of total PAHs formation in mutton kebabs at the heat treatment temperature of 250 °C, reaching 48.8 %, followed by LSE1.0 (43.2 %), LSE0.2 (33.2 %). Furthermore, the inhibitory effect on total PAHs formation enhanced with the increasing of LSE concentrations when the concentration of LSE was between 0.2 and 0.6 mg/mL. It was unexpected that the highest concentration group of LSE1.0 did not present the strongest inhibitory effect on total PAHs formation. Yu et al. (2023) confirmed that 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL of coriander root extract (CRE) and coriander leaf extract (CLE) treatments inhibited the formation of PAH8 (a sum of Chr, BaA, BbF, Bap, BkF, Bghip, Inp, DahA) in roasted duck wings. Among them, CRE at 0.6 mg/mL showed the best inhibition of PAH8 at 87.4 %, while CLE at 0.8 mg/mL performed best in reducing PAH8 at 51.8 %. However, Min et al. (2018) results showed a decrease in PAHs content with increasing epigallocatechin gallate (EGCG) concentrations in the range of 50–300 μg/kg. The reason for this discrepancy might be related to the small concentration range used in the latter study. If the concentration of EGCG is further increased, its inhibitory effect on PAHs may not continue to strengthen. This study suggested that LSE concentrations between 0.2 and 1.0 mg/mL had an inhibitory effect on the levels of total PAHs, but the levels of total PAHs did not always continue to reduce with increasing LSE concentrations. It was reported that longan seeds had a strong antioxidant activity, mainly due to their rich phenolic content, and among them, the main contributors to the antioxidant activity of longan seeds were 2 - hydroxy − 3 - methoxycaffeic acid 5 - *O* - β- D - glucopyranoside and 3′ - *O* - methyl – 4′ - *O* - (4 - *O* - galloyl -α - L - rhamnopyranosyl) ellagic acid (Chen, Xu, Ge, Zhu, Xu, & Li, 2015). LSE could effectively inhibit the formation of PAHs, and it was hypothesized that it interacted with the mutton kebabs, and the phenolic compounds attached to the surface of the mutton kebabs played a role in scavenging free radicals, thus blocking the cleavage, polymerization, and cyclization of PAHs during the baking process, which led to a decrease in PAHs levels. This interaction might also be saturated, then the inhibition of PAHs would not continue to increase.

In this study, the level and inhibition rate of each PAHs were different. The important contributors to total PAHs in the whole sample were Nap, Ace, Ant and Phe, while Bap, BkF, BaA, and Chr had lower contributions. Nap, which is the simplest PAHs with a structure consisting of two fused aromatic rings (Galushko, Minz, Schink, & Widdel, 1999). The contents of Nap in mutton kebabs treated with LSE0.2, LSE0.6 and LSE1.0 at 150 °C of baking temperature significantly decreased by 10.2, 19.5 and 16.4 % respectively in contrast with the control group ($P < 0.05$), 9.2, 20.9 and 16.2 % at 200 °C, respectively, and 17.4, 29.4 and 24.6 % at 250 °C, respectively. These findings were similar to the report of Hui et al. (2022), who found epicatechin could significantly inhibit the formation of Nap, and the inhibition range of Nap was 10–37 %. Ace, Ant and Phe, which are low molecular weight PAHs made up of three benzene rings (Naidoo & Naidoo, 2018). In comparison to the control group, treated with LSE (0.2, 0.6, 1.0 mg/mL) significantly reduced the levels of Ace, Ant and Phe in baked mutton kabobs at 150℃, and the inhibition range of Ace, Ant and Phe were 24.1–34.7 %, 17.9–43.6 % and 18.1–29.3 %, respectively, 32.5–57.0 %, 31.1–55.1 %, and 31.8–47.1 % at 200 °C, respectively, and 61.5–73.4 %, 31.9–53.8 %, 41.7–56.5 % at 250 °C, respectively. Hui et al. (2022) claimed that epicatechin could significantly inhibit the formation of Ant and Phe, and the inhibition range of Ant was 27–56 %, meanwhile, the Phe inhibition range was 9–100 %. Shen et al. (2022) confirmed that the addition of green tea extract, extract of bamboo leaves, grape seed extract and rosemary extract (5, 15, and 25 g/kg, respectively)

significantly reduced the levels of Ace, Ant and Phe in roasted duck skin. Bap, BkF, BaA and Chr, which are high molecular weight PAHs consisting of four or five benzene rings (Naidoo & Naidoo, 2018). In comparison to the control group, treated with LSE (0.2, 0.6, 1.0 mg/mL) significantly reduced the levels of Bap and BkF in baked mutton kabobs at 150℃, and the inhibition range of Bap and BkF was 14.3–22.4 % and 24.2–43.9 %, respectively, 15.2–22.8 % and 17.6–37.0 % at 200 °C, respectively. The inhibition ranges of Bap, BkF and BaA were 11.0–29.1 %, 22.0–46.4 %, and 15.2–30.4 % respectively at 250 °C. Likewise, Wang et al. (2019) found that treatment with quinic acid (0.05 and 0.075 mg/mL) significantly reduced the levels of BaA, BkF and Bap in charcoal- grilled chicken wings, and the inhibition range of BaA, BkF and Bap were 74.1–79.8 %, 48.0–54.0 % and 71.2–77.8 %, respectively. Furthermore, it was not that each PAHs was affected by LSE. For example, it had no significant effect on the production of Chr. Similarly, Wang et al. (2018) found that white tea had no significant effect on the production of BkF and DahA. LSE presented different inhibitory effects for each PAHs, which might be due to the fact that the differences in the molecular structure and the number of benzene rings of each PAHs, and the large differences in their chemical properties. Besides, the above reported additives and LSE showed different inhibitory effects on the formation of PAHs. This might be because different concentrations of additives had different levels of effective active substances. In addition, the structure of the phenolic substances was a crucial factor in the determination of their ability to scavenge free radicals, which influenced the kinetics of cyclization reactions generating PAHs, and different functional groups and structural properties have the possibility of conferring different antioxidant activities to phenolic substances. The results suggested that LSE inhibited the formation of PAHs in baked mutton kebabs, which might be attributed to the scavenging of free radicals by phenolic substances during the cracking and cyclization of hydrocarbon, thereby inhibiting the formation of PAHs, in addition to more complicated interactions such as combining with PAH intermediates (Onopiuk et al., 2022; Wang et al., 2019). In summary, LSE could effectively inhibit the formation of PAHs in baked mutton kebabs.

3.3. The lipid oxidation of baked mutton kebabs

The TBARS values represent the amount of MDA produced during lipid oxidation, which can reflect the level of lipid oxidation in baked mutton kebabs (Xia et al., 2021). As seen in Fig. 2, under the same concentration of LSE treatment, by comparing three groups with different cooking temperatures, the TBARS values in the baked mutton kebabs enhanced significantly as the temperature went up ($P < 0.05$). Heat treatment induced lipid oxidation in mutton (Roldan, Antequera, Armenteros, & Ruiz, 2014). Oz (2019) claimed that lipid oxidation deepened and TBARS values increased with the rising of heating temperatures. The TBARS values of baked mutton kebabs treated with LSE declined significantly in contrast with the control group. The TBARS values of baked mutton kebabs treated with LSE1.0 at different temperatures (150, 200 and 250 °C) of baking were significantly reduced by 34.1, 36.4 and 39.1 % in contrast to the control group, respectively. Cho et al. (2023) found that the MDA content of well-done pork patties added with 0.2 % and 0.4 % ethanolic extracts from perilla leaves significantly reduced by 85.4 % and 84.8 %, which indicated an inhibitory effect on lipid oxidation. Besides, Oz (2019) claimed that the TBARS values of beef meatballs added with 0.5 % and 1 % of black cumin significantly reduced by 11.4 and 16.7 %, which indicated an inhibitory effect on lipid oxidation. In this study, LSE decreased the levels of lipid oxidation, which might be due to its phenolic substances chelating transition metal ions and scavenging free radicals and carbonyl compounds (Zhu et al., 2022). In summary, LSE had an inhibitory effect on the lipid oxidation of baked mutton kebabs.

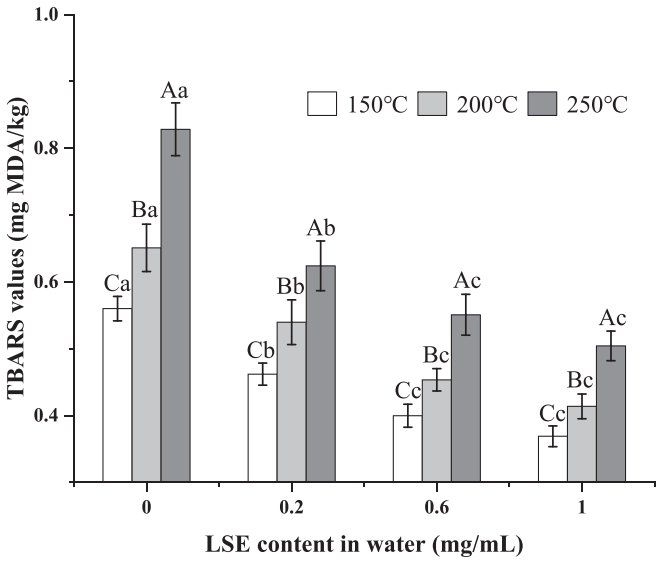

Fig. 2. The TBARS values of baked mutton kebabs at different concentrations of LSE and baking temperature. Data were expressed as mean ± standard deviation (n = 3). Results with different uppercase letters indicate significant differences between three baking temperature samples with same LSE content treatment at the level $P < 0.05$, and results with different lowercase letters indicate significant differences among the same baking temperature samples with different LSE content treatment at the level $P < 0.05$.

3.4. The protein oxidation of baked mutton kebabs

Carbonyl and sulfhydryl contents are broadly applied to estimate the levels of protein oxidation (Roldan et al., 2014; Xu, Zhu, Liu, & Cheng, 2018). The influence of different concentrations of LSE and baking temperature on the protein oxidation attribute of baked mutton kebabs was demonstrated in Fig. 3 and Fig. 4. The results showed that different baking temperatures affected the protein oxidation of mutton kebabs. By comparing three groups with different cooking temperatures, at the

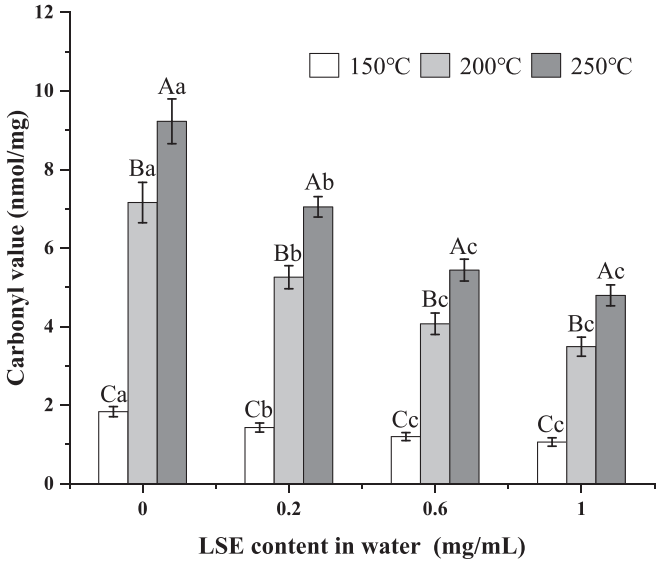

Fig. 3. The carbonyl contents of baked mutton kebabs at different concentrations of LSE and baking temperature. Data were expressed as mean ± standard deviation (n = 3). Results with different uppercase letters indicate significant differences between three baking temperature samples with same LSE content treatment at the level $P < 0.05$, and results with different lowercase letters indicate significant differences among the same baking temperature samples with different LSE content treatment at the level $P < 0.05$.

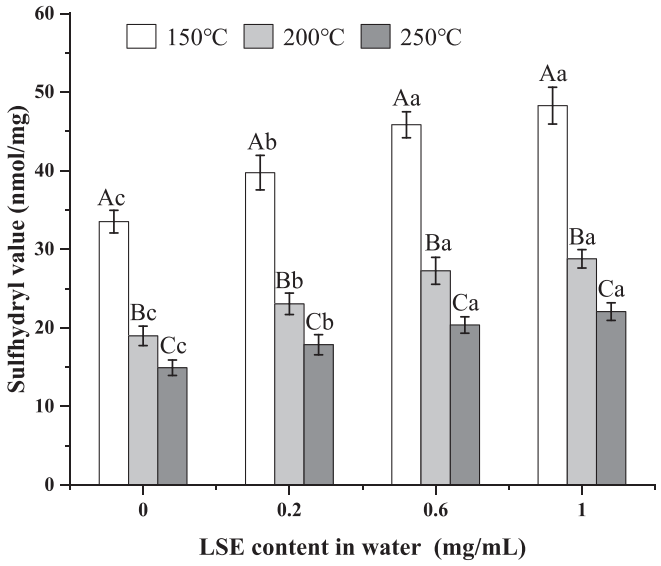

Fig. 4. The sulfhydryl contents of baked mutton kebabs at different concentrations of LSE and baking temperature. Data were expressed as mean ± standard deviation (n = 3). Results with different uppercase letters indicate significant differences between three baking temperature samples with same LSE content treatment at the level $P < 0.05$, and results with different lowercase letters indicate significant differences among the same baking temperature samples with different LSE content treatment at the level $P < 0.05$.

same concentrations of LSE, the carbonyl content of mutton kebabs enhanced significantly ($P < 0.05$) with the rising of baking temperature, and the sulfhydryl content decreased significantly ($P < 0.05$). Similar studies suggested that the rise in temperature during thermal processing accelerated the level of protein oxidation, which led to an increase in carbonyl content and a decrease in sulfhydryl content (Chen et al., 2023; Xia et al., 2021). The increased levels of protein oxidation might be due to the loss of antioxidant protection in muscle (Hoac, Daun, Trafikowska, Zackrisson, & Åkesson, 2006). In addition, heating probably caused the denaturation of myoglobin, and oxidative cleavage of heme pigments potentially resulted in the freeing of iron from the heme molecule, thus accelerating the oxidation of the protein (Traore et al., 2012). Contrasted with the control group at the same baking temperature, the LSE treatment could significantly reduce the carbonyl content of baked mutton kebabs, while the sulfhydryl content of mutton kebabs enhanced. Moreover, LSE reduced the degree of protein oxidation in concentration-dependent ways. When baked at 150 °C, the carbonyl contents of baked mutton kebabs treated with LSE0.2, LSE0.6, and LSE1.0 significantly declined by 22.0, 34.7, 42.1 %, and the sulfhydryl contents were significantly rose by 18.6, 36.8, 44.0 %, respectively. At 200 °C, corresponding decreased of 26.6, 43.1, 51.2 % and increased of 21.5, 43.7, 51.8 % were observed. At 250 °C, corresponding decreased of 23.6, 41.0, 48.0 % and increased of 19.6, 36.5, 47.9 % were observed. Zhao et al. (2021) confirmed that the protein carbonyl content of chicken meat with 0.6 % (w/w) *Adinandra nitida* leaf extract was reduced by 19.7 % and 19.7 % after frying at 170 °C and 190 °C, respectively. Fourati et al. (2020) discovered that addition of 1 % (w/w) pomegranate peel ethanol extract to minced beef sample reduced the carbonyl group accumulation and the disappearance of sulfhydryl proteins by 65.7 and 59.7 % respectively. Our results suggested that LSE reduced the levels of protein oxidation because its phenolic substances might act as scavengers of free radicals as well as chelators of metal ions (Zhu et al., 2022). Consequently, LSE could effectively inhibit the degree of protein oxidation in mutton kebabs during thermal processing.

4. Conclusion

The results showed that LSE could inhibit the formation of PAHs in baked mutton kebabs, moreover, the inhibition of PAHs in a certain concentration range increased as the concentration went up. The inhibitory effect of LSE on PAHs depended on its concentration and the baking temperature of the meat kebabs. Among them, the meat kebabs treated with LSE0.6 exhibited the strongest inhibition of PAHs when baked at 250 °C. Besides, LSE caused a significant decline in the TBARS value and carbonyl content and a significant rise in the sulfhydryl content of baked mutton kebabs. To summarize, the results showed that LSE could inhibit the formation of PAHs and muscle oxidation in baked mutton kebabs. This suggests that LSE has potential application in the food industry as a natural antioxidant and potential inhibitor to reduce the levels of PAHs when cooking food.

CRediT authorship contribution statement

Ruina Zhao: Data curation, Investigation, Methodology, Writing – original draft. **Yongsheng Zhang:** Project administration, Visualization. **Jingjing Chen:** Writing – review & editing. **Li Zhang:** Supervision, Visualization. **Cheng Chen:** Conceptualization, Investigation, Visualization. **Guoyuan Ma:** Supervision. **Xixiong Shi:** Conceptualization, Supervision.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100973.

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