



Effect of variable pressure-assisted immersion process using (–)-epicatechin on the color, flavor, and polycyclic aromatic hydrocarbons content in roasted beef meat

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are more easily formed in beef meat than other meats. A previous study confirmed the inhibitory effect of (–)-epicatechin on PAHs in a fat model system. However, (–)-epicatechin solution demonstrates colloidal properties at high concentrations. Hence a variable pressure immersion method was applied to infuse (–)-epicatechin in beef cubes. The fixed variable pressure-assisted immersion process using (–)-epicatechin that ranged from 0.2 mM/L to 5.0 mM/L was found to influence color, flavor, and PAHs dose-dependently. The 0.2 mM/L (–)-epicatechin treatment stabilized color properties, reduced PAHs content, and promoted the formation of volatiles and free amino acids. However, the use of more than 1.0 mM/L (–)-epicatechin, gradually decreased the values of brightness (L^*) and yellow to blue color (b^*), volatiles and free amino acids content, and reduced the inhibitory effect on PAHs. Therefore, the variable pressure-assisted immersion process of meat in 0.2 mM/L (–)-epicatechin can inhibit PAHs formation without compromising flavor properties of roasted beef.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of aromatic compounds formed by the fusion of two or more benzene rings. Most PAHs are carcinogenic and mutagenic. They can induce lung, stomach, liver, and other cancers *in vivo* (Kim et al., 2013). Diet is the major source of human exposure to PAHs (Phillips, 1999). Martorell et al. (2010) found that meat and meat products contributed to approximately one-half of the total contribution of each food group to the total PAH. Therefore, Commission Regulation (EU) 2020/1255 of 7 September 2020 amending Regulation (EC) No 1881/2006 had stated the maximum levels to be 5.0 µg/kg for benzo(a)pyrene and 30.0 µg/kg for the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene in traditionally smoked meat and smoked meat products, and traditionally smoked fish and smoked fishery products. Moreover, the levels of PAHs in processed meat products varied with different types of meat, such as beef, pork, lamb, chicken, and so on. PAHs levels in beef meat are more than mutton and pork meats (Onyang et al., 2012). High

temperature treatments have been found to yield the highest levels of PAHs in roasted beef, followed by roasted lamb meat and chicken meat (Moazzen et al., 2013). More PAHs are also generated in barbecued beef meat compared to barbecued pork and chicken meats (Aaslyng et al., 2013). PAHs level have been reported to be as high as 7881.60 µg/kg, 3484.0 µg/kg, 1781.4 µg/kg, and 691.20 µg/kg in roasted beef meat (Essumang et al., 2011), roasted pork meat (Rascón et al., 2019), roasted chicken meat (Wongmaneepratip et al., 2017), and roasted mutton meat (Akpambang et al., 2009), respectively.

PAHs are formed from the pyrolysis of lipids that are associated with free radical reactions (Hui et al., 2022). However, some PAHs can be formed from proteins and carbohydrates (Onopiuk et al., 2021). Epicatechin, a free radical and reactive carbonyl scavenger (Hui et al., 2021), can significantly inhibit the formation of hazardous compounds associated with lipid oxidation in roasted meat (Ding et al., 2021). The use of a chemical model system is an effective method of studying the effects of epicatechin on the formation of PAHs. A previous study that investigated the formation of PAHs in an intramuscular fat model system

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containing epicatechin demonstrated that epicatechin inhibited PAHs formation significantly (Hui et al., 2022). However, PAHs formation is much more complex in the actual meat matrix or system. Hence it is important to confirm whether epicatechin incorporated in roasted beef meat can inhibit formation of PAHs.

The antiradical nature of some polyphenols exerts strong antioxidant activity in meat products that can inhibit the carbonyl derivatives from lipid oxidation and protein oxidation (Xiong, 2017). These carbonyl species may also influence the formation of odor and taste in meat products (Boekel et al., 2006). Addition of some plant polyphenols, such as phytic acid and tea polyphenols when roasting chicken wings significantly inhibited the generation of flavor compounds that may result in negative effects on their sensory properties (Long et al., 2021). Some crude polyphenol extracts resulted in the decrease of free amino acids through the molecular reactions between polyphenol and amino acids, giving rise to the reduction of taste precursors (Noor-Soffalina et al., 2009). Inhibition of PAHs formation without compromising flavor properties is therefore an important consideration that determines consumer acceptance. To date, no research has investigated the effect of epicatechin addition on the flavor of roasted meats.

The incorporation of (–)-epicatechin in meat was found to be difficult due to the large size of beef muscle cubes, thereby reducing the antiradical nature or inhibitory effect on PAHs of (–)-epicatechin in roasted beef meat. This study for the first time employed a fixed variable pressure-assisted method previously used for salting of meat (Jiao et al., 2022) to incorporate (–)-epicatechin (0.2, 1.0, and 5.0 mM/L) into beef meat cubes. This method of incorporation helped improve the efficacy of (–)-epicatechin to inhibit PAH formation during the roasting of beef meat. Therefore, the aims of this study were to: I) investigate the effects of the fixed variable pressure-assisted immersion process with varying concentrations of (–)-epicatechin (0.2, 1.0, and 5.0 mM/L) on the color and flavor of roasted beef; II) investigate the effects of the (–)-epicatechin treatments on the formation of PAHs in roasted beef. This study provides a practical application of the variable pressure-assisted immersion process for the incorporation of (–)-epicatechin that can improve the safety and flavor quality of roasted beef.

2. Materials and methods

2.1. Chemicals and reagents

PAHs analytical standards purchased from Sigma-Aldrich (Shanghai, China) included phenanthrene, fluoranthene, pyrene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene, and benzo[a]anthracene that were stored at -20°C . Seventeen amino acid standards purchased from Sigma-Aldrich (Shanghai, China) were stored at -20°C . (–)-Epicatechin ($\geq 97.0\%$) was purchased from Beijing Solarbio Technology Co., Ltd. 2-methyl-3-heptanone (99%) and used as an internal standard for the determination of volatile compounds. *n*-Alkane standards (C7 – C40) used for the calculation of linear retention index were purchased from Jiancheng Technology Co., Ltd (Nanjing, China).

2.2. Materials

Six Chuannan mountainous cattle were obtained from the Mingshan Huacheng farm located at Ya'an City, Sichuan, China (East $102^{\circ} 58'$, North latitude $29^{\circ} 58'$, Altitude 588 m). The 26 months old cattle were fed in confinement with a commercial diet and transported to a local abattoir near the farm within 20 min from the laboratory. After 24 h of rest, the cattle were slaughtered according to the halal method following the principles and guidelines established by the Animal Care and Use Committee of the Sichuan Agricultural University, according to the National Research Council's Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). After chilling at 4°C and 87% humidity for 72 h, *longissimus thoracis et lumborum* muscles with an ultimate pH of 5.56 ± 0.35 on the right side of the carcass from the 6th

to 13th ribs were obtained according to the Chinese national standard GB/T29392-2012 "Beef cuts grading for high rib, ribeye, striploin, and tenderloin of national beef" (AQSIQ & SAC, 2012). After trimming of fat and connective tissue on the surface, these muscles were vacuum-packed with nylon/polyethylene, $9.3 \text{ mL O}_2/\text{m}^2/24 \text{ h}$, 0°C , 0.19 mm thick, Magic Seal®, Chengdu, China). Then vacuum-packed muscles were snap-frozen in liquid nitrogen and transported to the lab following cold-chain logistics within 6 h, and then stored at -30°C until further use.

2.3. Preparation of charcoal roasted beef

Figure S-1 shows the flow chart of the experimental design used in the present study.

2.3.1. Preparation of meat cubes and (–)-epicatechin solutions

The six *longissimus thoracis et lumborum* muscles were randomized. They represent the six replicate samples that were subjected to pressure-assisted immersion treatments using epicatechin in this study. Frozen *longissimus thoracis et lumborum* muscle was attached with a temperature probe and thawed in an incubator (Aikane Instrument and Equipment Co., Ltd., Shanghai, China) at 4°C until the core temperature reached -3 to -5°C . Then the muscle was cut into 96 cubes with dimensions of $4 \text{ cm} \times 4 \text{ cm} \times 1.5 \text{ cm}$ ($30 \pm 2 \text{ g}$). This cube size matches the dimension of Chinese traditional roasted beef cube. The 96 meat cubes were randomly divided into four groups, with twenty-four meat cubes in each group: epicatechin immersed pressure-assisted processed groups (E–0.2 mM/L, E–1.0 mM/L and E–5.0 mM/L) and control group (without epicatechin). (–)-Epicatechin (0.17, 0.87, or 4.35 g) were dissolved in 30 mL of ethanol (food-grade, National Standards of the People's Republic of China, GB 31640–2016) and made up to 3000 mL using distilled water followed by vortex magnetic mixing to prepare 0.2, 1.0 and 5.0 mM/L (–)-epicatechin solutions.

2.3.2. Variable pressure-assisted immersion process using (–)-epicatechin solutions

The variable pressure-assisted immersion method using (–)-epicatechin solution in this study was a modification of a variable pressure-assisted salting method used in a previous study (Jiao et al., 2021). Fig. 1 shows the schematic diagram of the dynamic varied pressure-assisted immersion equipment used. The equipment included the control valve, vacuum valve, buckles, clapboard, pickle barrel, display screen, vacuum system, and a refrigeration system. The prepared meat cubes were immersed in (–)-epicatechin solutions in a closed pickle barrel. The degree of vacuum was adjusted to alternate between atmospheric pressure and negative pressure to either incorporate the (–)-epicatechin solutions into the meat cubes or extract it. The back-and-forth movements of (–)-epicatechin solutions allowed sufficient penetration into the meat cubes. Temperature in the pickle barrel was set at 4°C , meat cubes immersed in brine at a ratio of 1:1 (w/w), curing time of 4 h, vacuum pressures of -70 to -80 kPa , and a pulsation ratio of 1.5 (vacuum/atmospheric, 54 min/36 min), when used with (–)-epicatechin solutions at concentrations of 0.2, 1.0 and 5.0 mM/L.

2.3.3. Charcoal roasting of the epicatechin-immersed beef meat cubes

The charcoal roasting procedures was carried out according to a previous method (Ding et al., 2021) with slight modification using a roasting equipment with a digital temperature sensor (SKJ-6082, LEPAIER, Shenzhen Lepaer Technology Co., Ltd). Charcoal roasted temperature was set between $500 \sim 600^{\circ}\text{C}$, with the distance of 10 cm between charcoal and roasted beef meat cubes. The roasted cubes were turned over once every minute. After 12 min of roasting, the core temperatures of roasted cubes were $85 \pm 2.5^{\circ}\text{C}$. Then the roasted cubes were cooled to $25 \pm 1^{\circ}\text{C}$ for 10 min. Immediately, for each group (twenty-four roasted beef meat cubes), the roasted cubes were randomly selected to be used for sensory (twelve), E-tongue (two), E-nose (two)

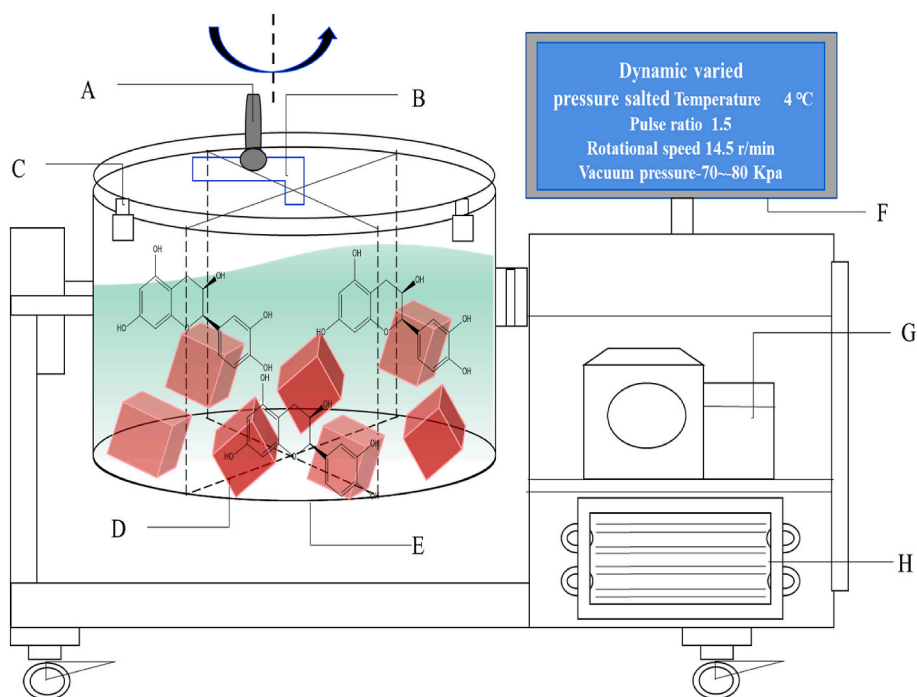


Fig. 1. The schematic diagram of the dynamic varied pressure-assisted immersion process used to incorporate (–)-epicatechin into meat cubes.

and color (two) evaluation. The remaining six cubes were randomly divided into three sub-groups (two cubes in each sub-groups) and further ground and vacuum-packed (stored at $-30\text{ }^{\circ}\text{C}$) prior to the analysis of volatile compounds, free amino acids, and PAHs, respectively. The roasted cubes were randomized for these analyses. The remaining five *longissimus thoracis et lumborum* muscles were prepared as described in the sections above. The days of pressure-assisted processing of muscles were randomized as well. Six replicate experiments were performed in the present study using the *longissimus thoracis et lumborum* muscles obtained from six animals.

2.4. Color measurement

Color parameters of the roasted beef cubes were determined using a chromameter (CR600d1, Konica Minolta, Inc., Tokyo, Japan) with illuminant D65, a 10° standard observer, and 8 mm aperture size using the CIE L^* (brightness), a^* (red to green color), b^* (yellow to blue color) color scale. The instrument was calibrated on a standard black and white enamel. The color measurement was performed perpendicular to the upper surface of roasted beef cube after blooming under daylight in air at a temperature of $20\text{ }^{\circ}\text{C}$ for approximately 25 min. L^* , a^* , and b^* values were analyzed by the Spectra Magic Software (Konica Minolta, Japan). The redness (a^*/b^* ratio) and saturation $[(a^{*2} + b^{*2})^{0.5}]$ indices were calculated according to Zajac et al. (2022). The results expressed as mean \pm standard error.

2.5. Determination of odor by E-nose

The procedure and analysis of odor of roasted beef was carried out using an E-nose (Xu et al., 2021). The E-nose (PEN3, Airsense, Germany) consisted of ten sensors S (type, performance, sensitive gases). S1 (W1C, aromatic compounds, methylbenzene), S2 (W5S, nitrogen oxides, nitrogen dioxide), S3 (W3C, ammonia and aromatic compounds, benzene), S4 (W6S, hydrogen, hydrogen), S5 (W5C, alkanes and aromatics, propane), S6 (W1S, methane, methane), S7 (W1W, sulfur compounds, hydrogen sulfide), S8 (W2S, alcohols, carbonic oxide), S9 (W2W, aromatics and organic sulfur, hydrogen sulfide compounds), and S10 (W3S, alkanes, methane).

One gram of grounded roasted beef was put into a 20 mL of headspace vial covered by a thin film, and held at 20 min at $25\text{ }^{\circ}\text{C}$. After that, volatile aroma in the headspace was transferred at a rate of 300 mL/min into the sensor system. The change in electrical conductivity (G/G0) of ten sensors was recorded per second, (G, electrical conductivity of sensor in contact with the sample; G0, electrical conductivity of sensor in contact with the gas filtered by activated carbon). The stronger the volatile aroma concentration, the G/G0 was farther away from 1, and if no aroma gas, G/G0 was 1. The measurement time was set at 60 s, and flush time was set as 90 s. A preliminary experiment showed that G/G0 was kept almost constant after 45s. The eigenvalues of G/G0 at 48, 49, 50, 51, 52 s were collected and calculated using the Winmuster software (Airsense, Schwerin, Germany).

2.6. Determination of taste by E-tongue

Roasted beef taste was determined using an E-tongue (Xu et al., 2021). The E-tongue (Astree, Alpha MOS, Toulouse, France) comprised of seven sensors and one reference electrode (Ag/AgCl). The AHS, CTS, NMS, ANS and SCS sensors represent sourness, saltiness, umami, sweetness, and bitterness, respectively, while the PKS and CPS sensors indicate complex taste compounds. Briefly, the roasted beef (40 g) was ground and then homogenized for 60 s with 200 mL distilled water. After that, the homogenized mixture was centrifuged at 8000 rpm for 15 min at $4\text{ }^{\circ}\text{C}$. The final supernatant was used for E-tongue analysis.

2.7. Determination of volatile compounds

The extraction of volatile compounds in roasted beef was carried out according to Xu et al. (2021). Two gram of the thawed ground roasted beef meat and $1.50\text{ }\mu\text{L}$ of 2-methyl-3-heptanone internal standard ($1.68\text{ }\mu\text{g}/\mu\text{L}$ in methanol) were put into a headspace vial (20 mL) for solid-phase microextraction. The vial was sealed and incubated at $55\text{ }^{\circ}\text{C}$ for 10 min. The volatile compounds are extracted with a SPME fiber coated with $65\text{ }\mu\text{m}$ polydimethylsiloxane/divinylbenzene (Supelco, Inc., Bellefonte, PA, USA) that was inserted into the vial headspace at $55\text{ }^{\circ}\text{C}$ for 45 min. The SPME fiber was then removed from the headspace vial and injected into the GC inlet to be desorbed at $250\text{ }^{\circ}\text{C}$ for 3 min. The

qualitative and quantitative determination of the extracted volatile compounds were carried out according to Xu et al. (2021) using a GC-MS system (QP2010 Shimadzu, Kyoto, Japan) that was fitted with a DB-WAX (30 m × 0.25 mm × 0.25 μm) column (Agilent Technologies, Santa Clara, CA, USA). Helium (99%) was used as a carrier gas with a flow rate of 1.50 mL/min. The initial temperature of the column oven was held at 40 °C for 3 min. Then the initial temperature was increased to 70 °C (2 °C/min), then further increased to 130 °C (3 °C/min), and finally increased to 230 °C (10 °C/min) where it was held for 10 min. The scanning range of mass spectra was 40–500 *m/z*, electron ionization mode at 70 eV. Ion source temperature was 200 °C, and transmission line temperature was 250 °C. Volatile compounds were identified using the NIST 2.0 mass spectrometry database and linear retention indexes (LRIs). The results were calculated relative to the internal standards and represented as ng/100 g samples.

2.8. Determination of free amino acids

The free amino acids (FAAs) content in roasted beef meat was determined according to Jiao et al. (2022) using an LA-8080 amino acid automatic analyzer (LA-8080 amino acid automatic analyzer, Hitachi, Co., Ltd., Tokyo, Japan). Four gram of the thawed ground roasted beef meat was used for FAAs analysis. The results were calculated relative to the internal standards and represented as mg/100 g samples.

2.9. Determination of PAHs

The determination of PAHs in roasted beef was carried out according to Hui et al. (2022) using a gas chromatography-mass spectrometry (GC-MS) (TRACE GC 1300 equipped with MS ISQ LT, Thermo Fisher Scientific Co., Ltd, USA) coupled with a HP5 column (30 m × 0.25 mm × 0.25 μm) (Agilent Technology (China) Co., Ltd.). The thawed ground roasted beef meat (0.5 g) was mixed with 2 mol/L sodium hydroxide solution (10 mL), and then heated at 85 °C for 1 h for full saponification. After heating, the mixture was cooled to 25 ± 1 °C. Then 5 mL of hexane was added and extraction carried out for 3 min, with the hexane phase collected after. This extraction procedure was further repeated twice and the hexane phases were combined. The collected hexane phase was dried with nitrogen at 25 °C. Then 1 mL of acetonitrile was added to redissolve, and filtered using a 0.22 μm filter (Keyilong Experimental Equipment Co., Ltd, Tianjin, China). The filtrates were collected for PAHs analysis. For GC-MS analysis, helium was used as carrier gas, with a flow rate of 0.7 mL/min. The initial temperature of column was set as 60 °C, held for 2 min; increased to 180 °C at a rate of 10 °C/min; increased to 200 °C at 5 °C/min and held for 3 min; and finally increased to 300 °C at 10 °C/min and held for 8 min. Single ion monitoring scan model was performed. Mass spectra was obtained by electron ionization at 70 eV. The temperature of mass spectra ion source, transmission line and injection were set as 200 °C, 250 °C and 290 °C, respectively. Splitting ratio was 10:1. The parent ion and three qualifier ions in the target PAH for qualitative analysis must be detected. PAHs were calculated by

comparing the retention time and their peak areas of standards. The results expressed as mean ± standard error (μg/kg). The linearity, detection/quantitation limits, and quantifier/qualifier ion of GC-MS assay for PAHs are shown in Table 1.

2.10. Statistical analysis

Results from six replicates were obtained in this study. One-way analysis of variance (ANOVA) was carried out using SPSS Statistics (Version 22.0, IBM Co., USA) to determine if significant differences ($P < 0.05$) exist between measures of color, volatile compounds, amino acids, and PAHs among the four different treatments. Least significant difference and Tukey multiple comparisons were performed if there were significant ANOVA differences. Descriptive statistics, homogeneity tests and estimates of effect size were performed with the confidence intervals of 95%. The results were expressed as mean ± standard error.

The discrimination of odor obtained using E-nose and taste using E-tongue for samples were performed by discrimination analysis (DA) using the XLSTAT software (Version 2019; Microsoft, USA) according to the method by Ding et al. (2021). The treatments were used as the dependent variables (X), the sensor probes in the E-nose or E-tongue were used as the explanatory variables (Y), and all replicates in all treatments were used as the observation labels. In model calculation, within-class covariance matrices are assumed to be different, prior probabilities are not taken into accounts, and significance was detected at the 0.05 level. The data from volatiles and free amino acids were also analyzed using principal components analysis and heat-map analysis using Origin Pro 2022b (Learning Edition), after normalized processing of data.

3. Results and discussion

3.1. Effect of variable pressure-assisted incorporation of (–)-epicatechin solution on the color of roasted beef

The roasted meat samples incorporated with 0.2–1.0 mM/L (–)-epicatechin solution by the variable pressure-assisted immersion method resulted in no significant ($P > 0.05$) effects on the L^* , a^* , b^* , and saturation compared to the control roasted beef meat (Fig. 2). This suggests that (–)-epicatechin treatment had no negative effects on the visual sensory attribute when its concentration was 1.0 mM/L and less. However, when the (–)-epicatechin treatment was incorporated at 5.0 mM/L, the values of b^* and saturation of roasted beef were significantly ($P > 0.05$) reduced compared to samples treated with 1.0 mM/L and less (–)-epicatechin. The solution of (–)-epicatechin, a type of polyphenol or condensed tannin (Haslam et al., 1989), has a light or off-white color under natural conditions, which did not make the color of raw beef meat undesirable. Phenolic hydroxyl compounds or tannin however are prone to oxidation in the presence of oxygen, which in turn results in the formation of brown-colored quinones (Shi et al., 2020). The oxidation of (–)-epicatechin can lead to the formation of brown-colored compounds,

Table 1

The qualitative, linearity, and limit of detection of PAHs using GC/MS analysis.

PHAs	T_R (min)	Intercept	Slope	R^2	Production (m/z)	LOD (ng/g)
Phenanthrene	13.49	−3927.3937	70815.3961	0.9993	178 ^{ab} , 179 ^b , 176 ^b , 152 ^b	0.10
Fluoranthene	17.27	−1308.6635	57794.4576	0.9991	202 ^{ab} , 203 ^b , 200 ^b , 101 ^b	0.06
Pyrene	18.33	−1137.5201	59779.6336	0.9995	202 ^{ab} , 203 ^b , 200 ^b , 101 ^b	0.06
Benz[a]anthracene	25.45	−6347.2251	30310.1661	0.9989	228 ^{ab} , 226 ^b , 229 ^b , 227 ^b	0.20
Chrysene	25.63	−211.7146	49208.5505	0.9987	278 ^{ab} , 279 ^b , 276 ^b , 139 ^b	0.07
Benzo[b]fluoranthene	30.53	−6483.5341	25926.3337	0.9985	253 ^{ab} , 252 ^b , 250 ^b , 126 ^b	0.40
Benzo[a]pyrene	31.57	−5382.9396	37457.2443	0.9987	252 ^{ab} , 253 ^b , 250 ^b , 126 ^b	0.17

T_R , retention time.

these values were estimated at a S/N ratio of 3.

a, quantifier ion.

b, qualifier ion.

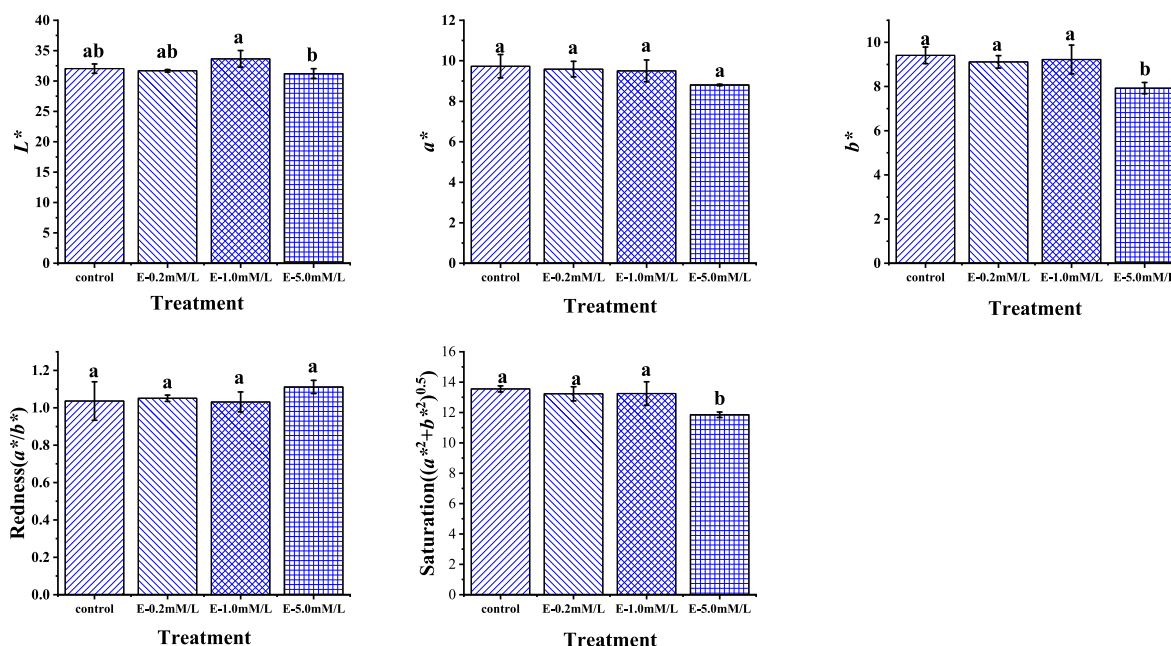


Fig. 2. Effect of (–)-epicatechin incorporation on the color of roasted beef. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

resulting in significantly reduce values of L^* and b^* at 5.0 mM/L concentration of epicatechin (Liu et al., 2010). Catechins, such as (–)-epigallocatechin gallate, (–)-epicatechin gallate, and their epimers, can participate in oxidation reaction between 100 and 165 °C in aqueous systems (Wang et al., 2006). It has been reported that the surface temperature of charcoal roasted meat was between 100 and 120 °C (Hui et al., 2022). This high temperature and oxygen might lead to the accumulation of brown pigment by the oxidation of (–)-epicatechin in the surface of roasted beef. This study indicated that when its concentration at 5.0 mM/L, the variable pressure-assisted immersion processing using (–)-epicatechin solution would change the color properties, and likely to decrease the L^* , b^* and saturation values ($P < 0.05$). However, at 1.0 mM/L or below (–)-epicatechin concentration, the color properties in roasted beef meat were not influenced significantly ($P > 0.05$) compared to control.

3.2. Effect of variable pressure-assisted incorporation of (–)-epicatechin solution on the volatile compounds and odor properties in roasted beef

Fig. 3a shows the principal component analysis loading plot of volatile compounds that had 55.37% and 38.40% of variance explained in the first (PC1) and second (PC2) principal components, respectively. Most volatiles had high positive loadings along PC1 that separated the control and E–0.2 mM/L treatments from E–1.0 mM/L and E–5.0 mM/L treatments. This clear separation along PC1 suggested that there were fundamental differences in the volatile composition with increased concentration of (–)-epicatechin solution. Fig. 3b shows the heatmap analysis of volatiles in roasted beef meat that clearly demonstrated the changes of individual compounds influenced by (–)-epicatechin. An obvious decrease in total volatiles occurred when the concentration of (–)-epicatechin solution was more than 1.0 mM/L. These volatile compounds included alcohols, aldehydes, ketones, and esters that are lipid oxidation-derived volatiles compounds (Hui et al., 2017). In general, such lipid oxidation-derived volatiles are quantitatively dominant, and contribute to the characteristic aroma of meat products (Toldrá, 2002). Epicatechin is a free radical and reactive carbonyl scavenger (Hui et al., 2021) that can inhibit formation of primary and secondary products from lipid oxidation (Shi et al., 2020). Epicatechin has been reported to be a natural antioxidant that showed protective effects on

lipid oxidation in beef meat (Jiao et al., 2019). These results suggested that the pressure-assisted immersion process using (–)-epicatechin solution from 1.0 to 5.0 mM/L mitigated the formation of characteristic aroma in roasted beef.

Meanwhile, E–0.2 mM/L treatment increased the aldehydes and ketones in the roasted beef meat, indicating a prooxidant activity for lipid derived volatiles (Tables S–1). Most plant polyphenols possess both antioxidant as well as prooxidant properties (Inoue et al., 1994). Azam et al. (2004) found that epicatechin was a naturally occurring antioxidant that also exhibited prooxidant properties. This prooxidant activity has been associated with epicatechin interaction with metal ions or other appropriate conditions. Autoxidation of (–)-epicatechin (Mochizuki et al., 2002) and its interaction with copper (Azam et al., 2004) led to the formation of superoxide and hydroxyl radical. Such radicals are important intermediates in the lipid oxidation radical chain reaction, which can induce oxidation of polyunsaturated fatty acid formation of hexanal, heptanal, nonanal, and other aldehydes, and some 2-ketones in meat products (Hui et al., 2017). Some phenolics after oxidation can be converted into quinones in aqueous solution state that act as reactive carbonyls (Zamora & Hidalgo, 2020), which is responsible for reaction flavors (Paravisini & Peterson, 2018). Shi et al. (2020) indicated that the capacity of polyphenol or tannin scavenging free radical or anti-oxidation function was correlated with their concentrations. When are present at more than 10^{-3} mol/L, they are antioxidants, while at below 10^{-5} mol/L they were prooxidants. Thus, (–)-epicatechin is dose-dependently an antioxidant or prooxidant. It can be inferred that (–)-epicatechin solution colloidal properties at high concentrations might inhibit its flow and subsequent reaction. However, at low concentrations, the aqueous solution state might favor its prooxidant activity. In general, aldehydes and ketones have low threshold values and play important role on meat characteristic aroma (Hui et al., 2017). This study indicated that the variable pressure-assisted immersion process that incorporated (–)-epicatechin solution at 0.2 mM/L might be helpful in maintaining the characteristic aroma of roasted beef meat.

Sensor arrays of the E-nose can distinguish odor or food flavors due to their high sensitivity and excellent selectivity. A slight change in volatiles would cause significant differences in sensor responses (Xu et al., 2021). Fig. 4a shows the loadings plot of sensors in E-nose in which PC1 and PC2 explained 59.48% and 27.19% of the variance

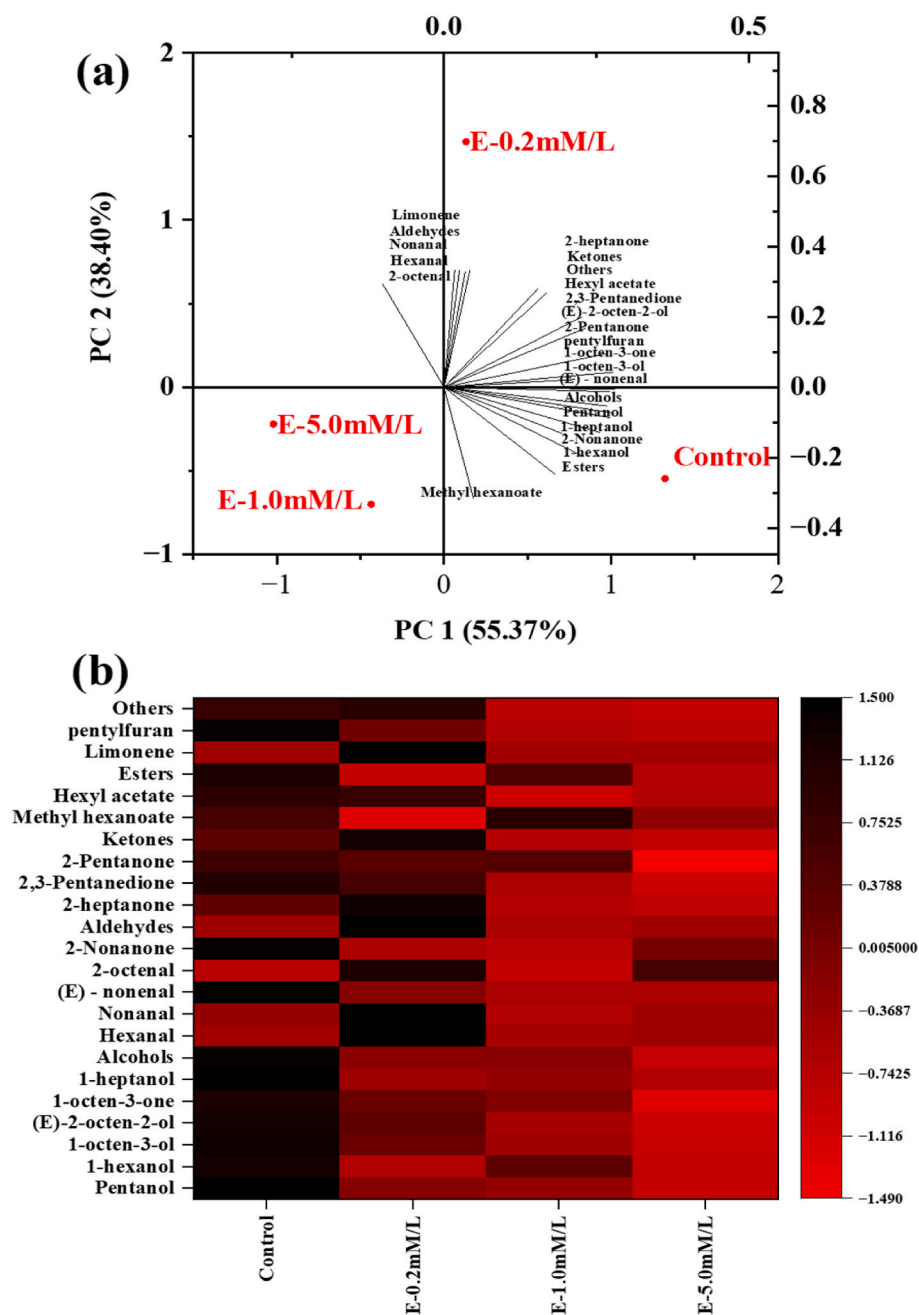


Fig. 3. Effect of (-)-epicatechin incorporation on the volatiles in roasted beef.

respectively. The scores plot separated the control, E-0.2 mM/L that had high negative scores from E-1.0 mM/L and E-5.0 mM/L treated samples that had high positive scores along PC1 (Fig. 4b). This indicated that the variable pressure-assisted immersion process of incorporating (-)-epicatechin at different concentrations resulted in differences in aroma. The control treatment was correlated to high negative loadings of W1S and W2S, while the E-0.2 mM/L treatment was correlated to a high negative loading of W1W along PC1. This clear separation along PC1 suggested that the roasted meat sample subjected to E-0.2 mM/L treatment had a similar odor profile to control meat sample. This result was consistent with volatile composition results (Fig. 3), which showed that the volatile profiles of roasted beef samples at low concentration (-)-epicatechin treatment was more similar to control roasted beef. On the other hand, the sample subjected to E-1.0 mM/L treatment was correlated to high positive loadings of W1C and W3C along PC2. Hence, the odor profile changed with increasing (-)-epicatechin solution

concentration.

3.3. Effect of variable pressure-assisted incorporation of (-)-epicatechin solution on the free amino acids and taste properties of roasted beef

Proteolysis is an important process for the release of free amino acids in meat products, particularly during the early stage of processing, such as curing, fermentation, and ripening (Toldrá, 2002). Proteolysis plays a crucial role on the taste attribute or sensory properties in meat products (Hui et al., 2017; Xu et al., 2021). Fig. 5a shows the PCA loadings plot of free amino acid that accounted for 54.49% and 28.92% of variance explained in PC1 and PC2, respectively. The meat sample subjected to E-0.2 mM/L treatment had high positive and control sample had high positive scores along PC1 that were correlated with more amino acids that had higher positive loadings. (-)-Epicatechin has antioxidant activity for meat protein in meat and meat products (Li & Ji, 2019; Rysman

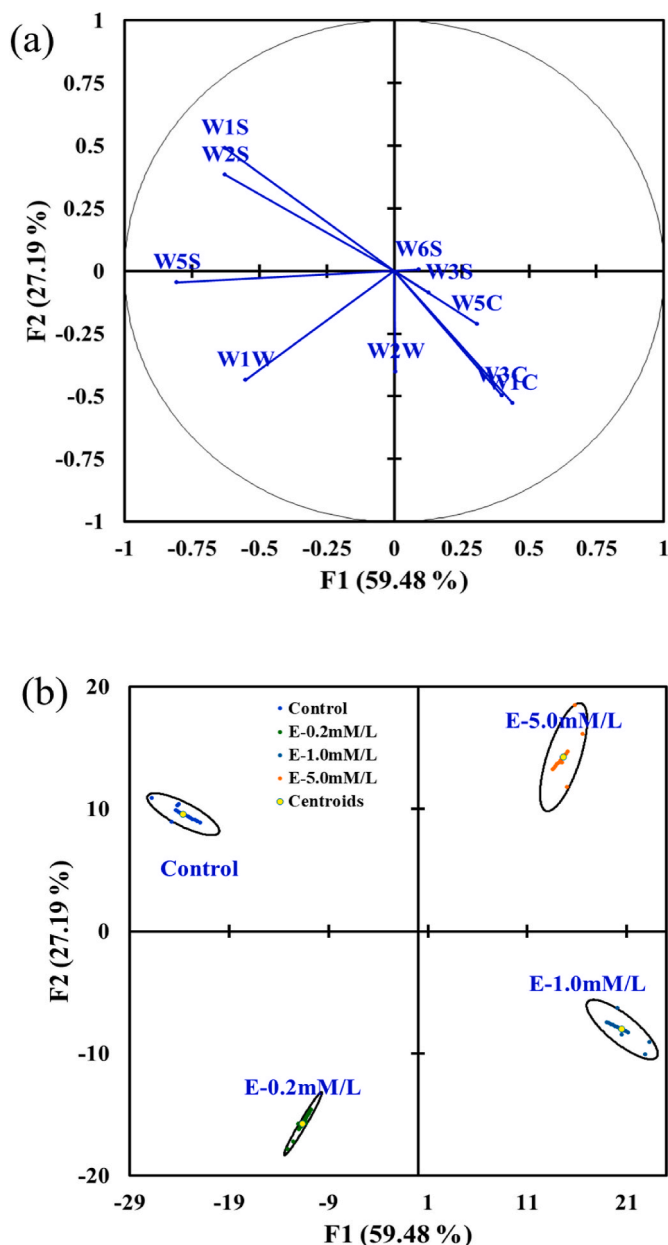


Fig. 4. Effect of (-)-epicatechin incorporation on the response of sensor probes in roasted beef using e-nose analysis.

et al., 2016). This confirmed function might play a protective role for protease in E-0.2 mM/L treatment thus favored the release of free amino acids compared to control treatment.

With high (-)-epicatechin concentration of E-5.0 mM/L, the roasted sample had high negative scores that were correlated to high negative loadings of some amino acids like cystine, glutamic acid, phenylalanine, proline, and isoleucine along PC1. This separation along PC1 suggested that the application of (-)-epicatechin resulted in obvious differences in free amino acids composition of roasted beef meat. The heatmap further showed the changes in individual free amino acids (Fig. 5b). Compared to the control group and meat subjected to E-0.2 mM/L treatment, the E-1.0 mM/L and E-5.0 mM/L treatments significantly resulted in a decrease of free amino acids. The occurrence of protein oxidation in roasted beef has been reported (Arcanjo et al., 2019; Li et al., 2021). The induction of protein oxidation can decrease the activity of proteases. In particular, the activity of cathepsin B + L, in a meat model system, resulted in diminished proteolysis and decreased

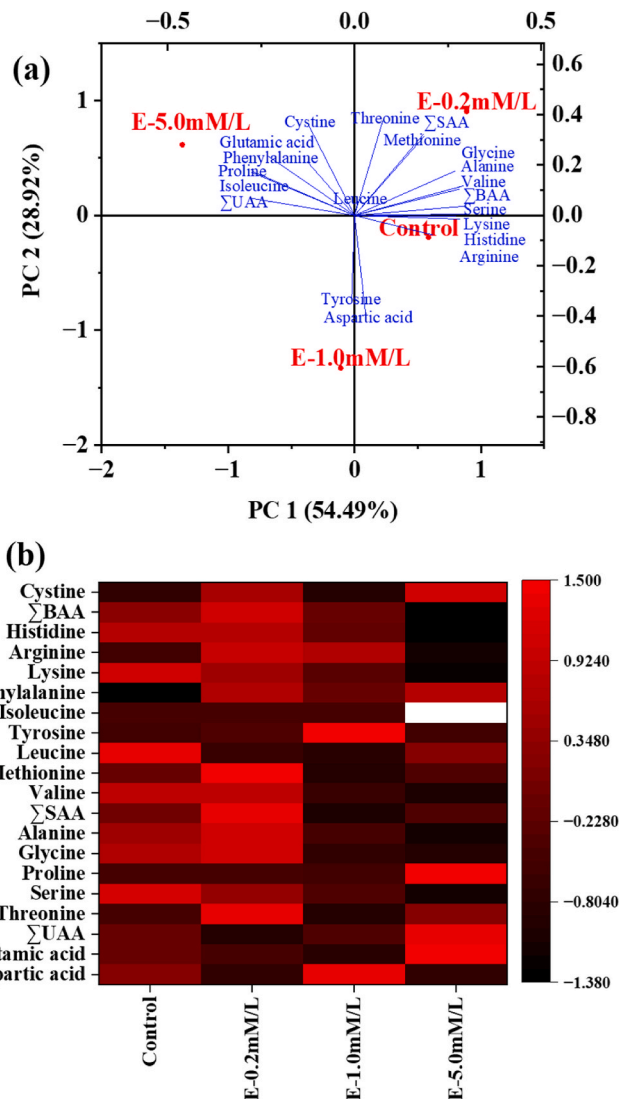


Fig. 5. Effect of (-)-epicatechin incorporation on the free amino acids in roasted beef.

amino acids (Berardo et al., 2015). Meanwhile polyphenols can be protease inhibitors through direct multi-hydrogen bonding and hydrophobic bonding that results in protease conformational changes (Shi et al., 2020). With increasing, (-)-epicatechin concentration, 1.0 mM/L and E-5.0 mM/L treatments might significantly reduce the catalytic capacity of protease through multi-cross links resulting in a decrease in free amino acids in roasted meat samples. A previous study reported increase in free amino acids in roasted meat after adding 0.02 mmol/100 g of (-)-epicatechin compared to control (Ding et al., 2021). However, with further increase in concentration from 0.11 to 0.56 mmol/100 g, the free amino acids contents decreased. Hence the incorporation of (-)-epicatechin will dose-dependently influence free amino acids release.

Sensor arrays of the E-tongue are highly sensitive and have been reported to distinguish taste changes in meat products (Xu et al., 2021). Fig. 6a shows the loading plot of E-tongue sensors where PC1 and PC2 explained 79.83% and 17.17% of variance respectively. The obvious separation in the scores plot indicated variable pressure-assisted immersed process by (-)-epicatechin solution had resulted in the significant change in taste attributes by influencing free amino acids release. The E-0.2 mM/L treatment had high positive scores along PC1 (Fig. 6b) that were correlated to a high positive loading of the ANS

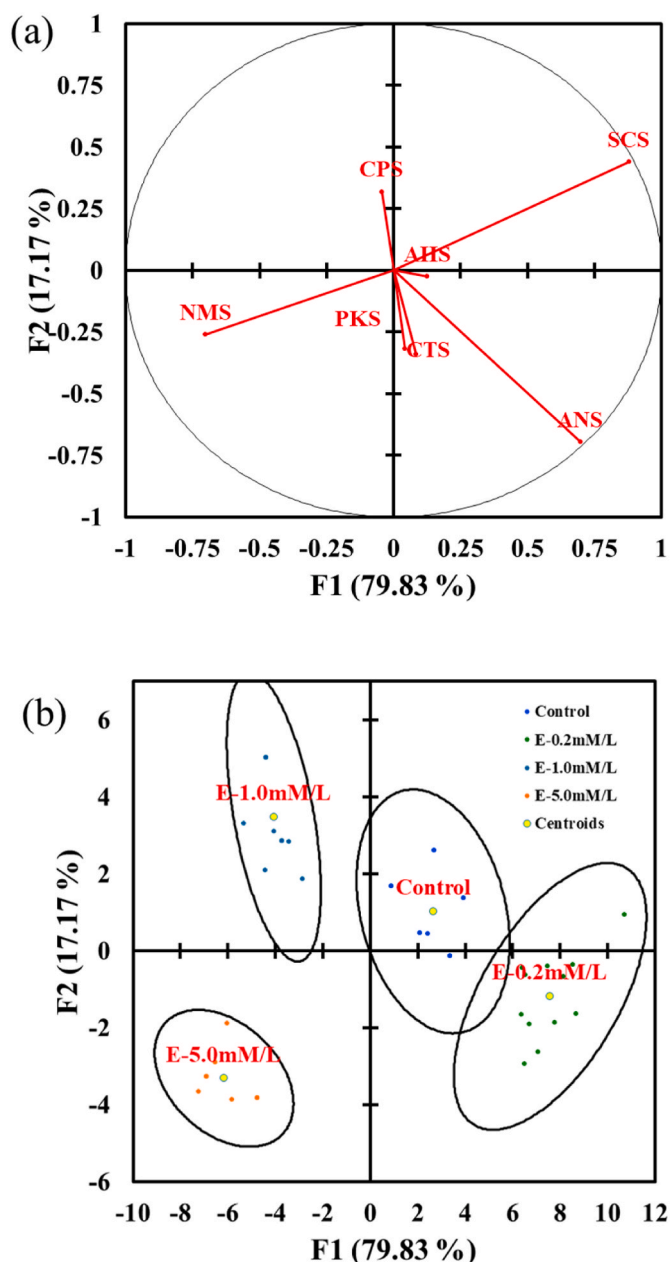


Fig. 6. Effect of (-)-epicatechin incorporation on the response of sensor probes in roasted beef using e-tongue analysis.

sensor (sweet), Interestingly, this sample was highly correlated to sweet amino acids that included alanine, glycine, serine, and threonine (Fig. 4a). However, with high (-)-epicatechin concentration, the E-5.0 mM/L treatment had a high negative score along PC1 (Fig. 6b) that were correlated to a high negative loading of the NMS sensor (umami), Interestingly, this sample was highly correlated to glutamic acid that contribute to umami flavor (Fig. 5a). These results show that amino acid compositions of samples were closely correlated to taste.

3.4. Effect of variable pressure-assisted incorporation of (-)-epicatechin solution on the PAHs in roasted beef

Fats or lipids was postulated to be the main precursors of PAHs as its formation involve a series of complicated chemical reactions that involve oxidative degradation, polymerization, and cyclization of fatty acids (Hui et al., 2022). The 0.1 mmol (-)-epicatechin addition in an intramuscular fat model system was found to inhibit formation of PAHs

formation (Hui et al., 2022). However, it is not known whether this inhibitory effect will occur in a complex meat system. As shown in Fig. 7, PAHs, phenanthrene, fluoranthene, pyrene, chrysene, benzo[b]fluorathene, benzo[a]pyrene, and benz[a]anthracene were detected in roasted beef. These PAHs were also detected in other beef meats (Rascón et al., 2019). From the results obtained, variable pressure-assisted immersion using (-)-epicatechin solutions reduced the total PAHs contents significantly ($P < 0.05$). These results indicated that besides chicken meat (Wang et al., 2019), (-)-epicatechin can be used to control PAHs formation in roasted beef and possibly other meats. Results also confirmed that epicatechin acted as lipid-derived radical and reactive carbonyl scavengers (Hui et al., 2021) that can effectively suppress PAHs formation in meat products. However, the effect of (-)-epicatechin incorporation in meat to inhibit PAHs formation was found to be dose-dependent. The best inhibitory effect was observed at 0.2 mM/L, where total PAHs level was significantly ($P < 0.05$) lower than control sample and other higher concentrations of epicatechin. The unsaturation and chemical structures of fatty acids can contribute to the diversity of their degradation products or intermediate products, resulting in the different PAHs formation (Chen & Chen, 2001; Hui et al., 2022). The different inhibitory rates of (-) epicatechin influenced the formation of all seven PAHs (Fig. 7). PAHs formation might be attributed to the pyrolysis of their specific fatty acid substrate(s) (Hui et al., 2022). The (-) epicatechin with multi-hydroxyl chemical structure might bind to specific fatty acid substrate(s) either covalently or non-covalently to form complexes. This may reduce different PAHs precursors and in turn results in different inhibitory rates. The binding of polyphenol to lipids has been reported (Boekel et al., 2006; Takechi & Tanaka, 1986). The properties of polyphenol solution are states between true solution and colloidal solution (Shi et al., 2020). When polyphenol concentrations are lower, the properties of solution become a true solution. However colloidal solutions are formed at higher polyphenol concentrations. The increased viscosity in colloidal solution can slow down the mobility of polyphenol molecules. This decreased mobility might mitigate the binding of (-) epicatechin to PAH precursors resulted in the generation of more PAHs in 1.0 mM/L and 5.0 mM/L roasted beef. Although Shi et al. (2020) indicated that polyphenols can reduce PAHs by directly binding with them to form a stable complex, the decreased mobility due to high polyphenol concentration may prevent this from happening. Therefore, the use of variable pressure-assisted immersion of incorporating (-)-epicatechin at low concentration can be useful to control formation of PAHs.

4. Conclusion

Techniques that can be applied to mitigate production of carcinogenic substances during processing of roasted meat without compromising flavor properties is important. This study for the first time employs a variable pressure-assisted immersion process using (-)-epicatechin solution to improve the safety and flavor qualities of charcoal-roasted beef meat. Results indicated (-)-epicatechin dose-dependently affected color, flavor, amino acids, and PAHs. Results confirmed that variable pressure-assisted immersion technique can be used to control PAHs formation in roasted meat. The 0.2 mM/L (-)-epicatechin treatment resulted in stable color, reduced PAHs contents, and conferred good flavor properties. Higher concentrations of (-)-epicatechin were found to reduce flavor quality and inhibitory effect on PAHs. Meanwhile, the variable pressure-assisted immersion technique helped improve the curing efficacy of (-)-epicatechin compared to static curing from traditional method, which can help to save energy. Future studies can be carried out using a myofibrillar protein model system to investigate the effect of (-)-epicatechin on protease activities and protein oxidation during heating. In addition, an intramuscular fat model system can be used to investigate the effect of (-)-epicatechin on reactive oxygen species and reactive carbonyl species.

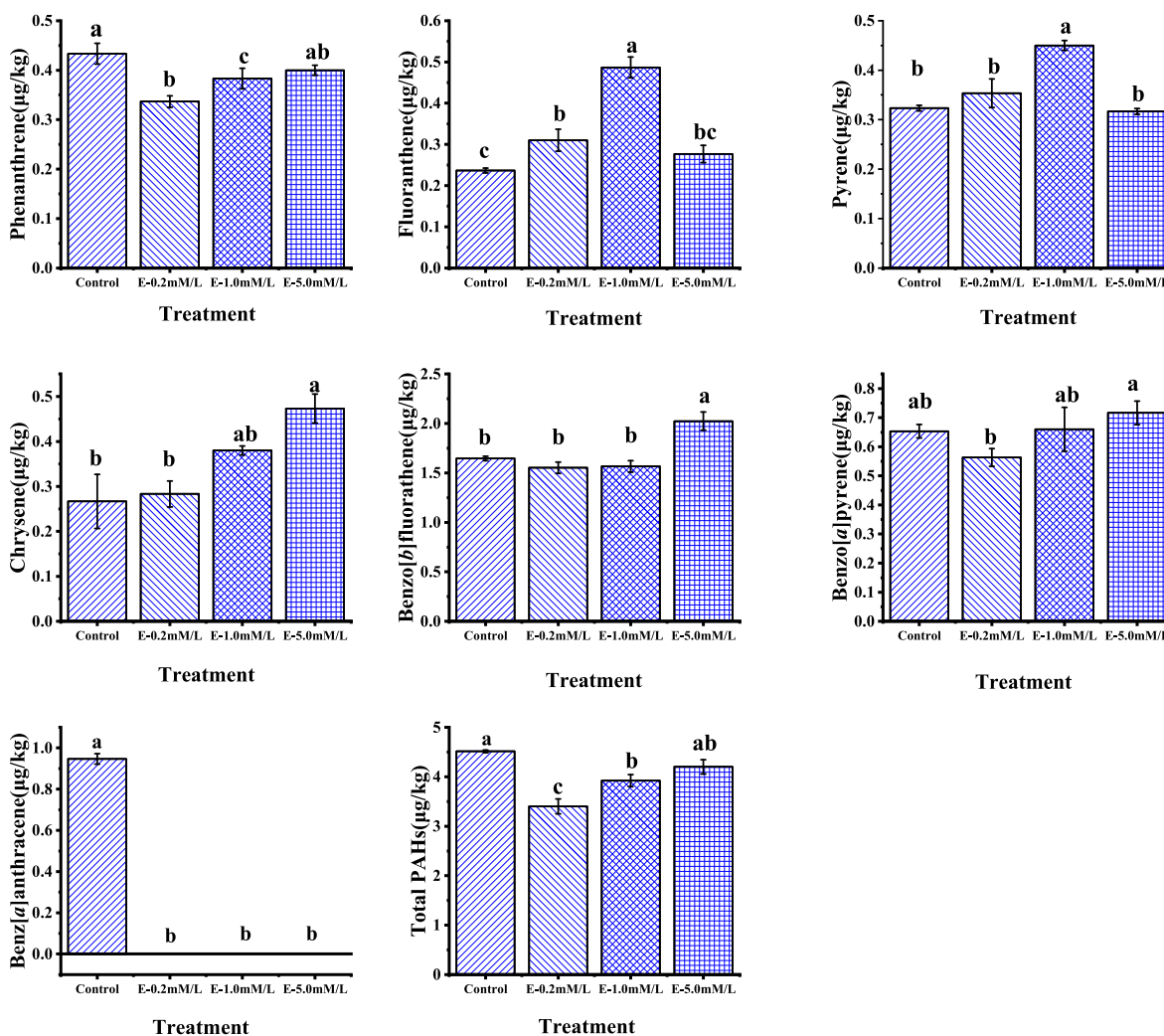


Fig. 7. Effect of (–)-epicatechin incorporation on the polycyclic aromatic hydrocarbons in roasted beef.

CRediT authorship contribution statement

Teng Hui: Writing – review & editing, Formal analysis, Conceptualization. **Zhengfeng Fang:** Conceptualization, Supervision. **Nazimah Hamid:** Writing – review & editing. **Qianli Ma:** Writing – review & editing. **Kezhou Cai:** Writing – review & editing.

Declaration of competing interest

The authors declare no competing financial interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2023.114602>.

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