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Semi-Quantitative and Qualitative Distinction of Aromatic and Flavour Compounds in Charcoal Grilled, Electric Barbecue Grilled, Infrared Grilled and Superheated-Steam Roasted Lamb Meat Patties Using GC/MC, E-nose and E-tongue

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Abstract: This study investigated the influence of four different methods of cooking (charcoal grilling, electric barbecue grilling, superheated-steam roasting and infrared grilling) on the volatile profile of lamb meat patties. The study included the patties of the oyster cut muscle of lambs cooked using charcoal grilling, electric barbecue grilling, infrared grilling and superheated-steam roasting methods. The principal component analysis (PCA) of electric nose data showed a total variance of 73.71%. The e-nose values showed differentiation of the volatiles released from the lamb meat patties. Data of PCA of e-nose and GC-MS revealed good separation between groups. Significance ($p < 0.05$) was found for the flavour profile values of charcoal-grilled and superheated-steam-roasted samples while the lowest significance was observed between infrared- and electric-barbecue-grilled samples. Saltiness and sourness were higher in superheated-steam-roasted patties than charcoal-grilled samples through e-tongue. The main volatile compound found in all the lamb patties was 2,3-octanedione with a content of 20.43 $\mu\text{g/g}$ –27.83 $\mu\text{g/g}$. 1-hexanol was highest at 34.74 $\mu\text{g/g}$ in the charcoal-grilled samples while 2,3-octanedione was highest at 35.83 $\mu\text{g/g}$ in superheated-steam-roasted patties.

Keywords: grilling; roasting; flavour; lamb meat; aroma

1. Introduction

The consumption of lamb in the world is estimated to be 2 kg per capita with different patterns of consumption between different continents. Mostly lamb is consumed and reared in countries such as Australia, New Zealand, Spain and China where there is a high production of lamb meat and where the lamb meat industry has been growing rapidly over the decades [1,2]. Lamb meat provides a source of nutrition to the consumers. Important nutrients (amino acids, etc.) in lamb meat help in many physiological functions of the human body such as protein digestion, tissue repair and building, fat metabolism, enhancing brain activity and physical strength [3]. According to the recommended daily intake, 100 g of lean meat of lamb provides 34 g/100 g of protein, 53 g/100 g of long-chain omega-3 fat as well as some important nutrients and minerals such as thiamine, riboflavin, niacin, sodium, phosphorus, zinc and potassium. A well-balanced consumption of lamb meat could play a significant role in our diet [4]. Consumer's attitude drives the eating quality of lamb meat regarding tenderness, sweet flavour and roast lamb taste developed in cooked meat [5,6]. Heat treatment of lean meat provides non-species and specific meaty

flavour while warming up meat containing phospholipids and triglycerides develops a species-specific flavour of meat. Thousands of volatile substances are produced during cooking, i.e., hydrocarbons, alcohols, ketones, esters, aldehydes, carboxylic acids, pyrans, lactones, pyrazines, furans, pyrroles, thiophenes, pyridines, phenols, thiazolines, thiazoles, oxazoles and other nitrogen and sulfuric compounds [7]. The species-specific flavour of meat is given by a combination of volatile composites which, in the case of thermally processed products, may contain even a few hundred compounds [8].

Water and fat in meat are influenced by cooking which ultimately impacts the taste of cooked meat. Aliphatic hydrocarbons and aldehydes were the main chemical families in cooked samples [9,10] identified 94 volatile flavour compounds in Bayannur roasted lamb. Among them were aldehydes, benzenoids, hydrocarbons, alcohols, ketones, esters, acids and other compounds, aldehydes and alcohols being the prominent compounds in all samples. The main flavour components of roasted lamb included nonanal, octanal, 1-octen-3-ol and hexanal. However, the temperature, cooking method and time of cooking determine the meat taste and flavour [11].

Different cooking methods (grilling, roasting and boiling) develop different kinds of flavour in lamb meat due to a reaction between amino acids and sugars as well as lipid degradation. This occurs at a temperature between 150 and 250 °C, and further denaturation of proteins on the surface of lamb mixed with the sugars creates the “meaty flavour” [12]. Fatty acids in lamb meat depend on cooking and produce volatile compounds upon cooking by lipid peroxidation. Therefore, the taste and aroma of cooked lamb meat are influenced by the fatty acid profile of the meat [13]. The powerful odorant compounds present in cooked meat are associated with the formation of carbonyl volatile compounds that result from oxidation of unsaturated fatty acids in lamb meat [14].

Ref. [15] reported a larger percentage of highly peroxidable polyunsaturated fatty acids in lamb grilled patties. They observed the decrease in the hexanal/3-methylbutanal ratio dehydration and surface darkening induced by heat suggesting the development of Maillard reactions.

Principal component analysis (PCA) allows one to select the most important volatiles influencing the aroma and flavour of lamb. The correlation between examined volatiles and aroma can be very useful for fast and objective evaluation of meat quality. In some studies concerning the volatile compound composition in meat, multivariate statistical techniques have been applied for data processing. Therefore, PCA for data visualization has been used to study the impact of different forage systems on the volatile composition of commercial lambs [16,17]. Ref. [16] applied PCA in order to summarise the relative differences amongst samples in relation to their overall fatty acid and volatile profiles in commercial lambs [16]. The first dimension of PCA explained 27.1% of the total variation in fatty acid and volatile composition, and the second dimension explained 18.2% of the total variation. On the other hand, Ref. [17] investigated the overall aroma profile in grazing lambs. According to the PCA results, the first two principal components explained 94.1% of the variance in aroma. They demonstrated that the feeding system had an influence on the volatile compounds of lamb meat. PCA analysis has also been applied to accurately identify the volatile compounds in beef processed by different treatments [18]. In this study, PCA showed good discrimination between the ultrasound treatment group and the control group since PC1 and PC2 accounted for more than 87% of the total variance [18]. Moreover, PCA has been used to clearly distinguish pig breeds according to their fatty acids and reheating volatiles [19]. These authors reported that five pig breeds could be clearly distinguished by PCA analysis according to their fatty acids and reheating volatiles. They observed that the Chinese indigenous pork may produce the lowest levels of the WOF during reheating compared with the four other pig breeds [19].

The multivariate statistical analysis was applied in some other studies on animal food products. Ref. [20] differentiated dry-cured hams and showed that the first two principal components explained 81.3% of total variability. The samples were clearly separated according to the progressive ripening time. Moreover, they stated that the volatiles profile

of Polish dry-cured ham had been intensively formed during all 30 weeks of ripening. Ref. [21] used PCA analysis in their studies on volatile compounds of Rhode Island Red (R-11) capons and cockerels. They observed that the proportion of the total variance explained by the first two principal components was 88.4%, and the classification accuracy, calculated using cross-validation, was 100%.

This study aimed to comprehensively analyse and quantify the different aromatic and flavour compounds in cooked lamb meat released upon cooking with different methods.

2. Material and Methods

2.1. Preparation of Lamb Patties

A total of 30 fresh lamb shoulder oyster cut muscles (oyster cut is the anatomical site of foreleg of lamb), from 8-month-old Bayannur sheep (Bayannur, Inner Mongolia, China), were obtained from Inner Mongolia Grassland Hongbao Food Co., Ltd. in Linhe, China. All animals had free access to pastures on the grassland in North China's Inner Mongolia Autonomous region. After slaughter, carcasses were kept at 4 °C for twenty hours, and oyster cut muscles were excised from carcasses, carved and kept at −80 °C until their further use. When used for experimentation and analysis, the lamb muscle packed in plastic bags was thawed with refrigerated water at 4 ± 1 °C for 24 h, and their core temperatures reached between −3 and −5 °C to avoid oxidative damage to meat. Fat was trimmed from the *oyster cut* muscles. Then lamb muscles were ground using a grinder with 5 mm blades to make lamb patties. Precisely 50 g of ground meat was used to make uniform patties using a Petri dish (6 cm × 1.5 cm).

2.2. Cooking of Patties

Lamb patties were cooked by four cooking procedures. Total number of patties for each treatment was $n = 30$. However, from each muscle, 10 patties were prepared, meaning that 3 muscles were required for the total 30 patties for each treatment. A total of 12 muscles were used for all 4 treatments to make $n = 120$ patties for all treatments. Charcoal-grilled patties were prepared on a charcoal barbecue grill for 10 min (5 min/side) to achieve a core temperature of 72 ± 5 °C, and the temperature (laser thermometer, Fluke, Everett, WA, USA) observed for charcoal fire grilling was 450–500 °C. For electric barbecue, the patties were cooked on an electric barbecue grill for 10 min (time achieved the core temperature) for all patties. For electric infrared grilling, the patties were grilled using an electric oven (Midea, Foshan, China) for the 17 min time required to achieve core temperature of 72 °C at 240 °C. For roasting the lamb patties, superheated steam was used with an electric oven (Midea) for 17 min time required to attain a core temperature of 72 °C at 240 °C (digital data logger; Zhejiang, Hangzhou Co., Ltd., Hangzhou, China). The thermometer probe was inserted into the geometric centre of each ground lamb patty. The lamb patties were cooled after cooking at room temperature (26 °C), packed in plastic ziplock bags and deposited at −20 °C until used.

2.3. Volatile Compound Profiling by Electronic Nose

APEN3 electronic nose system (Airsense, Schwerin, Germany) was used for acquisition and analysis of data. Ten sensors were placed in the measuring chamber of this piece of equipment (Win Muster Airsense Analytics Inc. Hagenower Straße, Schwerin, Germany). For loading the samples, 32 sites of automatic sampling apparatus (HSS32) were connected with automatic sampling apparatus [22]. Ground sample (2 g) was added in a sample vial of a 10 mL electronic nose. In order to eliminate humidity from the nearby environment, the inside headspace was calibrated for 1 h. Experiments were conducted at the temperature of 20 °C and 50–60% RH during all experiments, and the temperature was maintained constant with an accuracy of ± 1 °C. It was revealed in primary experiments that after equilibration of 30 min, the headspace reached a steady state. Before the program was run, the meat sample was heated to a controlled temperature (50 °C) for 5 min. The gas in the headspace was pumped out to the sensors of the sample electronic nose. During the

measurement process, phases were: stand-by (250 s), measurement (90 s) and concentration (10 s). The computer-program-controlled electric valves conducted the air through different circuits for each measurement phase. By measurement, the chamber airflow was kept persistent. During the phase of measurement, volatiles were pumped out by pump through the closed loop that involved the concentration chambers and measurement. In the loop, there was no flow of air. Measurement phase had 90 s duration, which was enough for stabilisation of sensors. A 250 s stand-by phase was activated after the completion of measurement for circuit cleansing and for the sensors' return to their baseline. Clean air circulated the circuit in this phase, first crossed the measurement chamber and after that empty concentration chamber, and the remaining volatiles were driven out of circuit. Each sample was repeated 7 times in parallel. The changes were experienced in resilience during measurement phases that were computer-recorded by sensors.

2.4. Analysis of Electric Tongue

An electronic tongue (Alpha MOS, Astree, Toulouse, France) with around seven sensors (SCS, ANS, CPS, NMS, CTS, PKS and AHS) and one reference electrode (Ag/AgCl) was used to analyse the taste attributes of lamb patties. Ground meat samples of fifty grams were homogenized with 200 mL distilled water and were centrifuged at $2265 \times g$ for about 10 min (Continent 512R, Hanil Corporation., Ltd., Guwangju, Korea). For the examination of electronic tongue by the obtained supernatants, sample temperature was maintained at temperature of 20 °C. The data were perceived as the pattern discrimination index in percentage and the patty taste attributes. These data were obtained using Alpha Soft program (Alpha MOS, version 19, ASTREE, Toulouse, France) [2].

2.5. Analysis by Gas Chromatography–Mass Spectrometry (GC–MS/MS)

In roasted *oyster* cuts, volatile constituents were detected as defined by [23] with some amendments. Extraction of volatile compounds was performed by headspace solid-phase microextraction (HS-SPME). Minced samples of two grams and 1.5 µL of 2-methyl-3-heptanone (prepared in 1.68 µg/mL in methanol) used as an internal standard were moved into a 20 mL headspace vial with stopper of PTFE silicon utilized in order to seal the vial. The vial was placed immediately into water bath and kept there for 20 min at 50 °C. After incubation, the volatile compounds from samples were absorbed using a coating fibre made of 65 µm divinylbenzene-fused silica/polydimethylsiloxane (DVB/PDMS) (Supelco, Inc., Bellefonte, PA, USA) by exposure of headspace vial for 40 min into GC equipment port, the coating fibre was immediately injected for 2 min for desorption. Using GC-MS (QP-2010 plus Shimadzu Corporation, Kyoto, Japan) with specification of attached DP-5MS (30 mm × 0.25 mm × 0.25 mm), the volatile substances were separated. At a constant flow mode, helium was distributed at 1 mL/min rate as the carrier. The temperature of the front inlet and ion source was 200 °C. The temperature of transfer line was 230 °C. The oven temperature was 40 °C kept for 3 min. It was increased to 120 °C for a time of 5 min and then to 200 °C maintained at 13 min. The mass spectra were with a mode of full scan of 40–500 m/z obtained at 70 eV. The component contents were quantified using 2-methyl-3-heptanone as an internal standard.

2.6. Statistical Analysis

Replicate experimental samples ($n = 5$) of cooked lamb meat of the four cooking methods were analysed in triplicate to investigate the effect of cooking method on the aromatic and flavour compounds released during cooking. Analysis of e-tongue, e-nose and GC-MS/MS was performed, and the obtained data were statistically analysed with application of SPSS (IBM SPSS Statistics, v.22; SPSS Inc., Chicago, IL, USA). The mean values were obtained. One-way analysis of variance (ANOVA) was applied to evaluate the effect of the cooking method on the aromatic and flavour compounds. In the case of significant effects ($p < 0.05$), Duncan's multiple range test was used ($p < 0.05$). A multivariate analysis approach was applied to the volatile metabolites detected using XLSTAT (Addinsoft, New

York, NY, USA). All volatile compounds from the e-nose analysis showing significant differences in the ANOVA analysis were included in a principal component analysis (PCA). Principal component analysis (PCA) and stepwise linear discriminant analysis (SLDA) were performed using the same XLSTAT program. These techniques were applied to the normalized relative amounts of the volatile compounds to reduce the dimensionality of the original data matrix retaining the maximum amount of variability allowing the visualization of the cooking treatment (volatile) in a two-dimensional space and identifying the directions in which most of the information is retained. For that, the original data were normalized, and the variables were classified into the first two components. Figures were prepared using Origin (v.2018, OriginLab Corporation, Northampton, MA, USA) statistical package.

3. Results and Discussion

3.1. GC-MS/MS Analysis of Volatile Compounds in Lamb Patties

The results of volatile compounds from the GC-MS/MS analysis are shown in Figure 1 in a heat map. Volatile organic compounds are formed through lipid oxidation, Strecker degradation or Maillard reaction [23], which are accountable for the development of meat flavour.

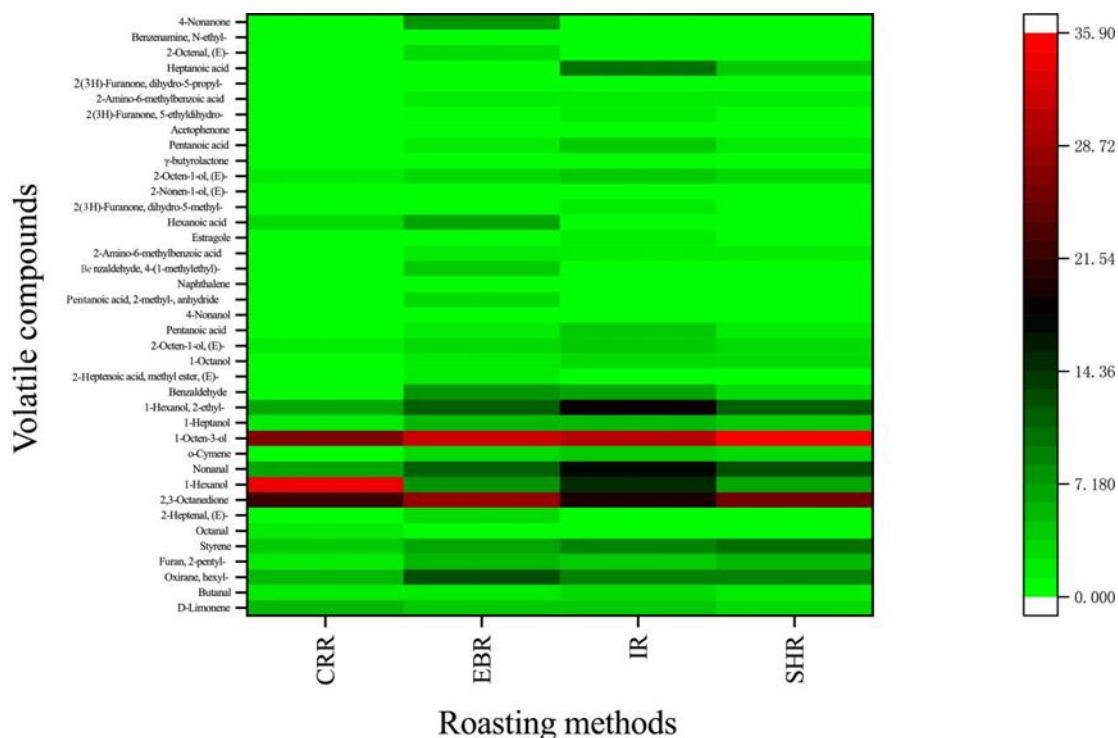


Figure 1. Heat map of total volatile compounds present in lamb patties cooked by four cooking methods. The abbreviations indicate CRR for charcoal-grilled, EBR for electric-barbecue-grilled, IR for infrared-grilled and SHR for superheated-steam-roasted.

The results of our study show the presence of 39 volatile compounds in lamb patties. 2,3-octadione is the compound most present with 20.43–27.83 $\mu\text{g/g}$ in all the different cooking methods. Superheated steam had the highest content of this compound. 1-hexanol was 7.02–34.74 $\mu\text{g/g}$ in all cooked samples. Charcoal-grilled samples had the highest content of 1-hexanol. 1-octen-3-ol was highest in superheated-steam patties with 35.83 $\mu\text{g/g}$, while in the charcoal-grilled sample, the content was 26.51 $\mu\text{g/g}$. Although the final temperature of the interior of the meat samples in all methods was 72 $^{\circ}\text{C}$, the time courses of the temperature in the four cooking methods were not recorded. The temperature in the interior of the meat samples could have increased at different rates

because of different heat transfer among cooking treatments. This could have influenced the odour and flavour of the lamb.

The meat volatile profile is dependent on many factors, the most important being the cooking procedure. Moreover, Ref. [24] suggested that 2,3-octanedione may be a biomarker for animals feeding on pastures. In this way, that compound helps to confirm the origin of the lamb meat.

It has been observed that the increase in cooking temperatures enhances the rate of chemical reactions producing compounds that provide the characteristic lamb aroma, this being the most relevant sensory quality for acceptance by lamb consumers. Ketones appear in large amounts in food and seem to have a great influence on the aroma giving a peculiar odour to meat and meat products [25]. However, it has recently been pointed out by [15] that some lipid-oxidation-derived volatiles and TBARSs increased while cooked-meat aroma compounds were reduced. According to [26], 2,3-octanedione can be considered as one of the important compounds distinguishing stir-frying stages. This compound has also been related to undesirable changes during meat product ripening due to microbial metabolism reactions [27]. However, meat from the present study was not aged; therefore the presence of 2,3 octanedione is more attributed to the cooking rather than to the ripening of meat. However, aldehydes appear to be more important to ketones among the volatiles in meat. Ref. [28] observed that the detectable volatile components in cooked meat are mostly generated from the oxidation of fat and volatile component production as they are affected by the cooking temperature and are greater for aldehydes (octanal, nonanal, benzaldehyde and hexanal) than for hydrocarbons (3-methylnonane, toluene, decan, benzene and hexane) and ketones.

On the other hand, it has been reported that 4-nonanol, 1-dodecanol, 1-hexanol, 1-pentanol and all long-straight-chain alcohols may contribute to miso product aroma with a lower threshold value [29]. In addition to that, branched-chain alcohols, such as 1-nonen-3-ol, (E)-2-octen-1-ol and 1-octen-3-ol, could participate in the aroma of the mutton dish *sao zi* as they also have low threshold values [26]. Aliphatic compounds might provide aroma to the meat flavour through unsaturated alcohols that have threshold values lower than those of saturated alcohols [29–31].

Meat proteins, which contain iron haemoglobin and myoglobin, are denatured during cooking liberating free iron which is a major oxidative catalyst in the meat, and endogenous antioxidant mechanisms of muscle are inactivated [32]. Afterwards, the free iron could promote interaction between substrates of unsaturated fatty acid and oxidants [33]. As a result of this interaction, aldehydes and alcohols appear as the most abundant volatiles in cooked lamb [13]. Other natural components that are present in muscle tissue such as hydrogen peroxide and ascorbic acid can also result in lipid oxidation promoting reactive oxygen species (ROS) formation or acting as catalysts.

Grilled lamb meat profile is created by the Strecker aldehydes, 2-methylbutanal and 3-methylbutanal [34]. However, we did not find these compounds in our samples. Linolenic acid (C18:3) degradation during cooking was reported to form benzaldehyde in cooked meat.

This is consistent with our results as we observed that benzaldehyde was present in the grilled samples as well as in other electrical cooking methods but not in open-charcoal-grilled samples; however, the content was lower in charcoal-grilled samples. The results of our study show heptanoic acid was the highest in infrared- and superheat-cooked samples with 9.96 µg/g and 4.16 µg/g, respectively, and hexanoic acid in charcoal- and electric-barbecue-grilled samples was found to be 3.35 µg/g and 6.59 µg/g, respectively. The report suggested that benzaldehyde produced burnt sugar and almond special flavour during processing [35]. Heating promotes rapid polyunsaturated fatty acid oxidation which produces a higher amount of free radicals capable of attacking those fatty acids less sensitive to oxidization such as oleic acid promoting the formation of aldehydes such as nonanal, octanal and heptanal [36].

Hexanal is the most important aldehyde compound in cooked samples representing between 87.30% and 90.80% of total aldehydes in the cooked meat. This finding is consistent with some studies which found that hexanal was the major aldehyde in the meat of cooked lamb [33] and goat [25] meat. However, we did not find this compound in our grilled or roasted samples.

Hexanal and 1-octen-3-ol are derived from the decomposition of C18:2n-6 fatty acid (linoleic acid) [34]. The cooking treatments of the present study most likely degraded linoleic acid further, thus, producing new volatiles and decreasing the hexanal content in the cooked lamb.

Hexanal has been more related to raw meat. Ref. [37] proposed the ratio of hexanal to nonanal as an indicator of raw lamb meat freshness and overall quality. A study by [13] on cooked lamb chop samples showed the detection of 74 volatiles identified by gas chromatography. The formation of these aromatic compounds is dependent on the concentration of lipids, amino acids and carbohydrates in raw meat [38]. Different chemical reactions utilize these substrates upon cooking with different methods and, most importantly, upon cooking conditions [39]. The most important and abundant odorant compounds found were hexanal, 2-ethyl-1-hexanol, 1-octene-3ol and octanal in cooked lamb and also consistent with other studies [14,40]. According to results by [23], hexanal was abundant in amount followed by benzyl alcohol, 1-octen-3-ol, nonanal and 2,3-octanedione with more than 1000 ng/g in samples from the burning charcoal lamb samples.

Associating gas chromatography data with odour and flavour is difficult. Few studies have quantified the contribution of these compounds to lamb flavour. Ref. [30] using partial least-squares models, reported that lamb flavour is the result of a complex balance among different odour-active volatile compounds. They observed that lamb flavour was positively correlated with the aroma concentration of 2-phenoxyethanol, acids and dimethylpyrazines and negatively correlated with Strecker derivatives, alkenals and alkadienals.

Recently, Ref. [41] identified the key odorants in traditional Chinese grilled mutton shashlik cooked on open carbon fire with or without suet (mutton fat) brushing during grilling. They identified a total of 57 odorants, which predominantly included pyrazines, sulphur-containing compounds and aliphatic aldehydes. However, they did not find the presence of (E,E)-2,4-decadienal as a key odorant.

We also found similar compounds in our cooked lamb patty samples, but there was no presence of hexanal; however, hexanol was present in our samples. The reason might be differences in cooking conditions and temperature requirements. In addition, the animal finishing system has also influence on lamb flavour. Ref. [42] reported that meat from lambs finished on pastures—without a concentrate supplement—had very low amounts of unsaturated aldehydes derived from lipids, ketones and Strecker aldehydes. The reason could be the protective effect of natural antioxidants in the diet. These results are consistent with TBARS values suggesting the development of lipid oxidation during cooking. Since we did not measure TBARS values, further investigation is therefore needed on this point.

Volatile compound content in lamb increases during storage time [42,43], but the meat from the present study was not stored or aged. That could explain the lack of aldehydes such as hexanal. Therefore the cooked meat in the current study could be characterised as lamb meat of low characteristic cooked lamb odour and flavour.

Branched-chain fatty acids (BCFAs) are not the only factor that influences juiciness, tenderness and flavour; other chemical compounds generated also effect consumer acceptance. Moreover, the overall sheep meat flavour and odour will be affected by other factors such as the ultimate pH of meat. It has also been reported that goat and mutton-like odours contain branched-chain fatty acid (BCFA), 4-methylnonanoic acid (MNA), 4-ethyloctanoic acid (EOA) and 4-methyloctanoic acid (MOA), along with octanoic acid, and thus during the cooking process, these compounds contribute to mutton odour. However, Ref. [44] suggested that reducing the effect of 4-methyloctanoic and 4-ethyloctanoic acid on the odour of grilled lamb will result in an improvement of the consumer acceptance of cooked meat. We found that 2-amino-6-methylbenzoic acid was present in our samples which

might contribute to cooked lamb aroma. In a study, Ref. [8] observed, a strong relationship between the intensity of the roasted flavour or cooked flavour of meat, amount of free amino acids, carnosine, pyrazines, hexanol and cooking temperature.

3.2. Volatile Analysis of Lamb Patties by E-Nose

An e-nose was used to assess the samples for distinguishing flavour based on information from a volatile analysis. PCA was used to analyse the e-nose data obtained from the cooked meat samples (Figure 2). The values for PCA were calculated from the data of sensors in the e-nose.

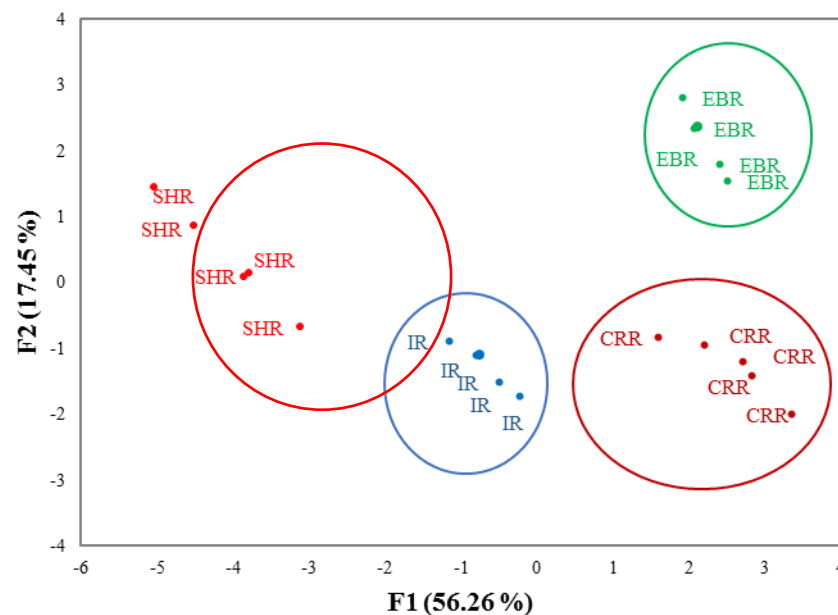


Figure 2. Principal component analysis score projection of e-nose volatile analysis of charcoal-grilled, electric barbecue-grilled, infrared grilled and superheated-steam-roasted lamb patties. The abbreviations indicate CRR for charcoal grilled, EBR for electric barbecue grilled, IR for infrared-grilled and SHR for superheated-steam-roasted.

From the analysis by the e-nose, it was observed that the first component PC1 showed 56.26% of variance, the second component PC2 showed 17.45% of variance, and the total variance was 73.71%. Figure 2 shows that there was a strong association between the e-nose volatile compound profile and the cooking method. This gives evidence that the volatile compounds generated by the patty cooking are closely related to the method of cooking. The results of the correspondence analysis show that the strongest correspondence to the volatile compound category was that of the electric-barbecue-grilled (EBR) and the infrared-grilled (IR).

The discriminant analysis (Table 1) showed that all cooking types had good discrimination ($p < 0.05$) between each other with the exception of charcoal-grilled and electric-barbecue-grilled samples of lamb patties where discriminant values were not significant ($p > 0.05$). These two cooking methods are also in the same part of PC1 which could mean that both of them distinguish or explain equally the e-nose volatile compounds.

Table 1. Discrimination analysis of volatile compound profile among the different cooked lamb patty samples using e-nose.

Header Cooking Treatments	Discrimination Power			
	Charcoal-Grilled	Electric Barbecue	Infrared-Grilled	Superheated-Steam-Roasted
Charcoal-grilled		0.461	0.628 *	0.765 *
Electric barbecue			0.753 *	0.794 *
Infrared-grilled				0.613 *
Superheated-steam-roasted				

* values in the same column bearing different superscript letters differ significantly ($p < 0.05$).

In a study by [45], a PCA analysis showed that the cumulative variance was 97.90%. Both PC1 and PC2 helped to distinguish the flavour profile of the mutton that was processed. The results show that there existed a correlation among the flavour components within the samples based on the volatile compound distinction. The content and nature of the volatile organic compounds (VOCs) that develop after cooking are influenced by genetic (sex, selection, breed, species) factors, meat processing, feeding and animal management and product storage. Different breeds of sheep or lamb also have an impact on different volatile compound formation while cooking.

The ability of the e-nose in differentiating volatiles released from cooked lamb meat samples by different methods was evaluated using discrimination power values derived from pairwise PCA analysis (Table 1).

Discrimination power was expressed as a decimal fraction of the proportion of principle component identification of the lamb patties' elements that were identified and determined to be different among cooking methods. Table 1 shows that among the four cooking methods, the most significant difference ($p < 0.05$) was observed between electric-barbecue and superheated-steam-roasted samples.

Lipid oxidation is also an important process that enhances the development of volatile compounds. As a result, about 50% of volatile components generated are lipid oxidation products. The volatile compounds identified in the present study agree with the volatile profiles reported for lamb fat and low-temperature (<90 °C) heated meat [14].

Few cyclic compounds were formed such as alkenes that indicate a stronger heat treatment. Therefore, higher heat generation will produce higher amounts of cyclic compounds. Cooking at high temperatures leads to oxygen discharge and hence production of iron inducing free radicals. Therefore, cooked meats are even more susceptible to lipid oxidation than raw meat. In addition, cooking also stimulates the release of non-heme iron from pigments as a result of the phospholipid exposition to oxygen. The increase of non-heme iron release is faster in slow heating than fast heating. Reduction of breakdown of hydroperoxide into free radicals and lower activation energy for oxidation have been associated with high-temperature cooking [46].

Cooking at moderate or low temperature (80 °C for 6 h, 60 °C for 6 and 24 h) resulted in a high production of volatile compounds from lipid oxidation while a more severe temperature and time combination (80 °C for 24 h) arose at a higher concentration of 2-methylpropanal and 3-methylbutanal [9] originated from Strecker degradations in the amino acids. Therefore, cooking at high temperatures for long times results in stronger meaty flavour and roast notes in lamb meat formation.

Finally, the low temperature used in the cooking methods of the current study resulted in low amounts of Strecker degradation compounds that account for the strong meaty flavour. Therefore, we could state that the meat of our study had low lamb odour and flavour.

3.3. Flavour Analysis of Lamb Patties by E-Tongue

The PCA analysis shows the contribution rate of the accumulative variance of principal components 1 and 2 (Figure 3). This contribution is greater than >90%, indicating that there

is a lot of flavour information. Moreover, there is also high significance ($p < 0.05$) among the samples cooked by four cooking methods. Infrared grill and superheated steam were located almost in one plot while the charcoal-grilled and electric-barbecue-grilled were located in two different plots. This shows that superheated-steam-roasted and infrared-grilled samples had a significant ($p < 0.05$) positive correlation with each other. There was a non-significant positive correlation between charcoal-grilled and electric-barbecue-grilled samples. However, there was a significant negative correlation ($p < 0.05$) between the charcoal-grilled and the superheated-steam-roasted samples. A study by [25], through PCA analysis with a total variance of 90%, showed that the lamb had a significant distribution of volatile compounds contributing to the overall flavour profile with a total variance of 90%.

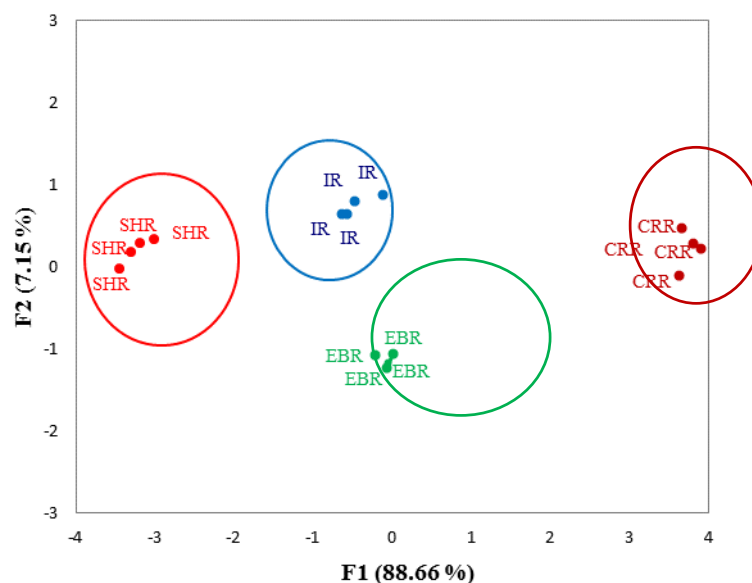


Figure 3. Principal component analysis score projection of e-tongue flavour analysis of charcoal-grilled, electric-barbecue-grilled, infrared-grilled and superheated-steam-roasted lamb patties. The abbreviations indicate CRR for charcoal-grilled, EBR for electric-barbecue-grilled, IR for infrared-grilled and SHR for superheated-steam-roasted.

Table 2 shows the response scores of the flavour analysis in the lamb patties. The results of the electric tongue analysis show that all the four cooking methods produced significantly ($p < 0.05$) different effects on the lamb patties’ flavour.

Table 2. Changes in the responses of electronic tongue sensors in lamb meat patties cooked by four methods.

Cooking Method	AHS-Sourness	PKS	CTS-Saltiness	NMS-Umami	CPS	ANS	SCS
Charcoal grill	2.9	8.7 *	3.6	3.1	9.0 **	8.7 *	9.0 **
Electric barbecue	6.0	6.3	5.0	6.0	5.9	4.0	6.1
Infrared grill	6.6	5.7	6.7	6.4	5.4	7.2	5.4
Superheated steam roast	8.5 **	3.3	8.7 **	8.6 **	3.7	4.2	3.5

*, ** values within the same column differ significantly (* $p < 0.05$; ** $p < 0.01$).

The sourness score was highest (8.5) in the superheated-steam-roasted lamb patties. It was lowest (2.9) in the charcoal-grilled patties. There was a significant difference ($p < 0.05$) in the flavour of these two types of cooked patties. The saltiness was seen to be significantly ($p < 0.05$) highest in the superheated steam (8.7) while lowest in the charcoal-grilled patties (3.6). Moreover, the umami flavour was also seen to be significantly highest $p < 0.05$ in the superheated-steam-roasted sample while others had a lower score than this method of cooking.

The comparison of the scores of flavour analysis (Table 2) could indicate that the better heat transfers in the superheated-steam oven favoured the formation of flavour compounds (2,3-octanedione and 1-octen-3-ol) compared to the charcoal grill. In addition, those flavour compounds might be related to the stronger sourness, saltiness and umami flavours in the lamb cooked in the superheated-steam oven. However, undesired characteristics have also been associated with 2,3-octanedione formation [20].

Our results of the study are consistent with [47] where all these flavour attributes (sweet, salty, umami and sour) were found in the grilled lamb loin steaks. According to [33], there was a barny taste attribute reported which is expected in lamb meat due to skatole or manure taste. However, we did not find this taste attribute in our analysis.

The score of flavour sensors was highest in superheated steam than the other methods of infrared-grilled, electric barbecue and charcoal-grilled. Dry heat cooking methods have different effects compared to moist heat cooking methods. Time is a very important factor in the grilling and boiling of steaks and kebabs. Many previous studies have shown that in roasting, many flavouring compounds are lost such as pyrazines, thiazoles and oxazoles [8]. Moist heat cooking methods work on different phenomena using a varied amount of liquid to preserve flavour at various high temperatures and different times. In Table 3, distance analysis between the cooking methods shows the finger print identity index and the distance.

The higher the value of distance, the higher the significant difference ($p < 0.05$) is among the two cooking methods. As observed in Table 3, the superheated steam and the charcoal-grilled compared had the highest significance with a distance value of 798.21. Second were charcoal and infrared grill with a distance value of 491.29. The lowest distance value 149.41 was observed in the comparison between the electric and the infrared-grilled samples which showed the least significant difference ($p < 0.05$). The reason between these two methods for flavour is the difference in cooking methods. The moist heat methods produce natural flavour in tender cuts by solubilizing the collagen, and the steam produced by these methods converts the tough collagen into tender and soft gelatine [8].

Table 3. Distance analysis of two cooking methods in lamb patties among the four cooking methods by electric tongue.

Sample Names	Comparative Sample	Distance	<i>p</i> -Value	Finger Print Identity Index (%)
Charcoal grill	Electric barbecue	436.09 *	0.00	94.46 **
Charcoal grill	Infrared grill	491.29 *	0.00	95.55 **
Charcoal grill	Superheated steam	798.21 **	0.00	98.29 **
Electric barbecue	Infrared grill	149.41	0.00	62.39
Electric barbecue	Superheated steam	385.20 *	0.00	91.36 *
Infrared grill	Superheated steam	313.71 *	0.00	88.01 *

*, ** values within the same column differ significantly (* $p < 0.05$; ** $p < 0.01$).

ANOVA analysis of the e-tongue showed that the *p*-value of the cooking method was less than 0.05 indicating that the cooking method has a significant effect on value. Similarly, sensors also have *p*-values lower than 0.05. This means there is a 0% chance of obtaining values by random variation. As far as the cooking method and sensor interaction effect is concerned, the interaction effect was also significant ($p < 0.05$).

We reject all three null hypotheses and accept an alternative hypothesis that there is a highly significant effect of the cooking method, sensors and their interaction on values. The value of R^2 (coefficient of determination) shows that 100% variation in values is explained by the cooking method, variables and their interaction.

During cooking, nutrients such as proteins, sugars and vitamins suffer complex reactions that include degradation pathways to produce specific odour, flavour and taste compounds. The amount and intensity of these compounds account for different lamb tastes depending on the heat treatment. During moist heat cooking, the flavour compounds might dissolve into the cooking liquid producing delicately flavoured meat. However, the amount of water used in a specific cooking method makes a significant difference among

the moist cooking methods and also with the dry cooking methods inducing flavour in the meat [48]. Table 4 shows that the cooking methods individually have significant differences from the other three methods.

Table 4. Multiple-comparison test between the cooking treatments for e-tongue analysis.

(I) Cooking Method	(J) Cooking Method	Mean Difference (I-J)	Standard Error	Significance.
CRR	EBR	130.91 *	6.46	0.00
	IR	128.49 *	6.46	0.00
	SHR	215.32 *	6.46	0.00
EBR	CRR	−130.91 *	6.46	0.00
	IR	−2.41	6.46	0.70
	SHR	84.41 *	6.46	0.00
IR	CRR	−128.49 *	6.46	0.00
	EBR	2.4182	6.46	0.70
	SHR	86.83 *	6.46	0.00
SHR	CRR	−215.32 *	6.46	0.00
	EBR	−84.41 *	6.46	0.00
	IR	−86.83 *	6.46	0.00

The error term is Mean Square (Error) = 584.976. * The mean difference is significant at the 0.05 level.

The two methods electric barbecue and infrared-grilled do not have a significant difference ($p > 0.05$) between each other. Flavour is a basic characteristic in the evaluation of meat. During and after the procedure of cooking, an important number of chemical reactions between precursors, degradation products and intermediate reaction products gives place to many compounds in the meat. Some of these compounds modify the meat flavour and can be dispersed in air [38].

Therefore, the odour-active compounds related to the cooking method not only contribute to lamb taste but also have a significant influence on lamb flavour through their interactions with both intrinsic constituents of meat and subsequent products of degradation produced by cooking.

4. Conclusions

The study concluded that the aroma and flavour of lamb meat were affected by the cooking methods. Among the four cooking procedures, there existed a significant change in the aromatic and flavour profiles. The reason might be due to heating temperature and the method of cooking used. The use of dry heat cooking methods such as charcoal grilling had a different impact on the volatile profile of lamb meat as compared to superheated-steam roasting. Moreover, superheated steam had a higher content of the volatile compounds while charcoal and other methods had fewer compounds, which suggests that due to higher temperature, some important volatile compounds might be lost upon heating.

Principal component analysis of GC-MS and e-nose data showed satisfactory separation between groups. With different cooking methods, the protein, amino acids and flavour compounds in lamb increased contributing to the typical flavours of the product. The different temperature applied during lamb cooking may be the critical contributor to formation of varied volatile flavour compounds at different cooking time points. From the e-nose results, it can be concluded that cooking lamb meat patties at low temperature resulted in low amounts of Strecker degradation compounds that account for the strong meaty flavour. In addition, few cyclic compounds were formed such as alkenes indicating that heat treatment was not very strong. Therefore, the meat of our study had low lamb odour and flavour. Further investigation is needed to understand the biochemical changes that could govern the aromatic profile modifications in cooked lamb meat.

Author Contributions: R.S. and T.H. performed the experiments and wrote the whole manuscript after analysis and review of the literature. Z.W. checked the results, verified the data and helped in the scientific editing of the manuscript. A.D.A.-R. critically revised the whole manuscript and gave suggestions for improvement of the draft. H.L. helped in data analysis with table and figure formation, and D.Z. critically revised the whole manuscript with technical editing and improving the aim of the manuscript. All authors have read and agreed to the published version of the manuscript.

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