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Identification of volatile compounds in several meat and bone broth using Solid Phase Micro Extraction-Gas Chromatography Mass Spectrometry (SPME-GCMS) for initial detection of Halal and Non-Halal Food

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ABSTRACT

The development of techniques for detecting halal and non-halal animal meat is of great importance as a useful tool for monitoring and assuring food quality for Muslim. Flavor or aroma is one of the sensory attributes for consumers to distinguish broth cattle. The differences aroma of broth cattle is contributed by the content of volatile compounds. This study was aimed to determine the composition of volatile compounds in meat and bone broth from five different animals, i.e., cow (beef), pig (pork), goat, lamb, and chicken for initial identification of halal and non halal food. A preliminary test for samples of meat and bone broth was done by identifying their physical properties including pH, viscosity, and density. The volatile compounds in meat and broth samples were extracted using a solid phase-micro extraction (SPME) at room temperature and analyzed by gas chromatography-mass spectrometry (GCMS). The results revealed the physical properties of meat and bone broth, i.e., pH, viscosity, and density, cannot be used to distinguish the type of broth produced from beef, pork, goat, lamb, and chicken. Successful results for the identification were achieved through chromatogram profile of volatile compounds from meat and bone broth which show characteristics to each type of animal, and therefore it can be used to distinguish pork from beef, goat, lamb, and chicken as initial detection for halal and non halal food. The research also identified 7 predominant volatile compounds as a marker for meat pork and other 8 specific compounds marker for pork bone.

Key word: volatile compound, halal, meat, bone, broth, SPME, GC-MS

INTRODUCTION

The increase of Muslim population give rise to the demand for halal foods. Based on the Islamic shariah, pork is a non-halal meat that is prohibited from being consumed by Muslim [1]. However, due to economic reasons the adulteration of meat and meat product with lower-priced pork meat has become a global issue. Therefore, the development of analytical methods capable of distinguishing pork meat from other different cattle is crucial. The meat and bone broth are commonly used as a gravy or flavoring in a variety of foods. The water-soluble components in broth produce flavors and aroma which is characteristics to the different type of animal; however, no research has been done to use this type of sample (meat and bone broth)

as material to distinguish halal and non-halal identification. Based on Millard reaction, under thermal processing, reducing sugars and amino acids in broth induce the formation of the flavor-intensive components. Besides, unsaturated fatty acids, as well as aldehyde-fatty-acid components, also contribute to the formation of odorous heterocyclic flavor compounds through fat degradation process and plays an important role by giving a distinctive taste and aroma of certain parts of animal [2]. Thus, different source of meat or bone from different kind of animals may lead to produce different volatile compounds and is expected can be used to distinguish pork broth from other sources, such as beef, goat, chicken, and lamb broth, and the elucidation of the volatile compounds of different types of animal broth is also useful to gain a better understanding of flavor generation [3].

A common method for identification of this type of halal food is based on polymerase chain reaction (PCR), real time-polymerase chain reaction (RT-PCR), and protein-based methods using enzyme linked immunosorbent assay (ELISA) [4,5]. However, those techniques require rigorous sample preparation, costly reagents, and is time-consuming. Numerous instrumental methods have also been reported for meat products authentication based on the volatile compounds in pork [6-8], beef and pork [9-13], lamb [14-19], goat [20], and chicken [1,21-22].

Determination of aroma compounds in pork broth using the solid phase microextraction (SPME) was reported. The aroma compounds were dominated by aldehydes, alcohols, and ketones [6]. Analysis of volatile compounds in pork bones showed 7 volatile compounds, including hexanal, octanal, 6-methyl-5-hepten-2-one, 2-nonanone, decanol, benzaldehyde, and (E)-2-nonenal. Other volatile compounds found in pork bone are based on butyric acid, octane, and indole [7]. Volatile compounds that play an active role in giving aroma to pork from other report are pentanal, 3-hydroxy-2-butanone, hexanal, 2-hexenal, 2-methyl-3-furantiol, heptanal, 3-(methylthio) propanal, 2-heptenal, dimethyl trisulfide, octen-3-ol, 2-ethyl-3-methylpyrazine, nonanal, 2-nonenal [8]. The volatile organic compounds, including 2,3-butanedione, 3-methyl butyraldehyde, 3-methyl-1-butanol, and acetoin, produced from the pork stored in refrigerators at 4°C, had a positive correlation with the pork freshness [9].

Identification of volatile compounds in meat broth has reported the difference between beef and pork broth in the concentration of their constituents. Beef contains higher levels of 2-furfurithiol, 2-methyl-3-furanthiol, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone than pork [10]. Volatile organic substances determined by HS-SPME–GC–MS in raw and cooked beef were different and could be clarified into hydrocarbons, alcohols, aldehydes, acids, esters, ketones, furans, and S-containing compounds [11]. The volatile compounds in cooked Irish beef are dimethyl sulfide, 2-butanone, ethyl acetate, 2-methylbutanal, 3-methylbutanal, 2-heptanone, dimethyl trisulfide, and nonanal [12]. The volatile compounds from cooked marrow bone broth were analyzed using gas chromatography-mass spectrometry (GC-MS) after solid phase microextraction (SPME) [13].

Some papers have reported the volatile profile of lamb meat and volatile branched-chain fatty acids. A large number of volatile compounds are produced during lamb stewing that contribute to the overall aroma of stewed meat [15]. Meanwhile, special taste and aroma of lamb meat was reported to be affected by the composition of volatile compounds. Identification of volatile compounds in lamb meat using SPME with GC-MS instrumentation had found 52 volatile compounds consisted of aldehyde, alcohols, ketones, alkanes, alkenes, aromatic, heterocyclic, furan, sulfur, ester, and terpenes compounds [16]. In other research using the similar method, 17 volatile compounds, including alcohols, aldehydes, heterocyclic, benzene, hydrocarbon, sulfur, and ether compounds were obtained. Aldehydes were volatile compounds that dominate animals [17].

The HS-SPME-GC/MS coupled with chemometrics, based on the relative intensity spectral data, found ninety-seven volatile compounds from lamb meat. The largest classes of volatile compounds were aldehydes and furans followed by alcohols, hydrocarbons, ketones, sulphur and nitrogen compounds [18]. Analysis of volatile compounds from lamb meat with different dietary treatments using HS-SPME-GC/MS has also been reported. The volatile compounds such as sulphur compounds, aldehydes, alcohols, ketones, terpenes, phenols, indoles, pyrazines, furan, benzenoid compounds, hydrocarbons, and organic acid were insufficient to impact highly sensory quality [19]. Moreover, the volatile compounds in goat meat extracted using the SPME/GC-MS showed 153 compounds, and some of these compounds play an active role in giving aroma. Aroma-active compound from stewed goat meat by GC-MS/olfactometry resulted 26 compounds contributed as aroma-active, such as nonanal, 2-octenal, and 2,4-decadienal, dimethyl trisulfide, 3-methyl-butanal, octanal, 2-decenal, 2-nonenal, methanethiol, hexanal, 2-undecenal, and 1-octen-3-ol [20].

Technique of GC-MS/olfactometry was used to investigate the difference between two types of chicken broth, broiler broth (BB) and native chicken broth (NCB). The result showed that NCB contained more complex volatiles and exhibited a richer aromatic profile compared with BB. NCB contained 2,4-decadienal, 2,4-decadienal, 2,4-nonadienal, 2-nonenal as the aromatic compound with the highest flavor dilution (FD) factor in both broths [1] [21]. Ten key favor substances, such as 1-phellandrene, linalool, myrcene, limonene, ocimene, anethole, terpineol, dipropyl disulfide and 2,4-decadienal, were investigated [22]. However, research which investigate the differences volatile compounds in various kinds of broth has never been carried out.

All of the above volatile compound analysis methods which employed SPME to collect volatiles in the headspace (HS) combined with GC-MS provided information most closely matching the reality [23]. SPME is a green analytical technique because it does not use organic solvents, thus it is considerably harmless and environmentally friendly. This method provides several advantages, including free extract from solvent impurities, relatively short extraction time, lower possibility of analyte loss, and easy integration with GC instruments [24]. Moreover, SPME gives a better estimation of the aroma profile as perceived by the human nose [25]. SPME direct injection method is simpler since the sample is in direct contact with the SPME fiber [26]. This special fiber can be used up to 1000 times for analysis and can be integrated easily with GC or GC-MS without requiring modification of GC or GC-MS [24] [26], thus it is cost-effective.

Therefore, this research is aimed to determine the composition of volatile compounds in meat and bone broth from beef, pork, goat, lamb, and chicken using HS-SPME combined with GC-MS. The research was started by investigating the physical properties of meat and bone broth from all type of animal including pH, viscosity, and density, followed by analysis of volatile compounds content in meat and bone broth among different animals. The characteristics aroma of broth from different type of animal which presumably contains different composition of volatile compounds is expected to give different profile of chromatogram and can be used to distinguish broth of the five type animals. Moreover, this study is also expected to obtain specific compounds in meat and bone pork broth; thus, this finding can be used as pork broth markers for halal detection method as a contribution to science in supporting halal and non-halal food identification.

EXPERIMENT

Materials

Pork was obtained from *Sus scrofa*/local pig, beef was obtained from local cow *Bos taurus* / *Sapi Madura*, lamb is obtained from local sheep (*Ovis aries*) / *domba ekor gemuk*, goat was obtained from *Domestic Goat* (*Capra aegagrus hircus*) / *kambing kacang* and chicken is obtained from *gallus domesticus Neornithes/broiler chicken*. All samples were obtained from *Rumah Potong Hewan* (RPH) Malang City, except chicken were from *Rumah Potong Unggas* (RPU) Batu City. The beef, lamb and goat were slaughtered in a slaughterhouse according to standard procedures from *Majelis Ulama Indonesia* (MUI). All meat was taken from foreleg/thigh part, while the bones were taken from the rib or rough part. Thermo Scientific Orion pH buffers standard solution 4.01, 7.00, 10.01, Acetone (E-Merck), and Aquabidest Waterone.

Instrumentation

The equipment used in this work included oven (Memmert UF 55), analytical balance (Mettler Toledo), SNB-2 Viscometer, Millipore Sigma™ Supelco™ SPME Fiber Assembly, Divinylbenzene/Carboxen™/Polydimethylsiloxane (DVB/CAR/PDMS), df 50/30 µm. Identification of volatile compounds using GC-MS QP2010 column Rtx-wax (30m x 0.25mm x 0.25 µm), Thermo Scientific™ Eutech™ pH 150 Meter, and Glassware from Pyrex.

Procedure

Preparation and Physical Test

Broth preparation

One hundred grams of each meat and bones was added with 200 mL of water in a 500 mL flask and closed tightly with a blue lid (temperature resistant thermoplastic polyester allowing them up to 180°C). The samples were heated in the oven at 100°C for 3 hours. The broth was cooled down to room temperature and filtered off using Whatman filter paper no. 41. The broth obtained was tested for their pH, density, and viscosity.

Determination of pH, viscosity, and density

The pH, viscosity, and density of each meat and bone broth from pork, beef, goat lamb, and chicken were determined directly to all the broth using a calibrated pH meter, SNB-2 Viscometer, and pycnometer respectively with three replications.

Solid phase microextraction of volatile compounds from broth

A carboxen-poly dimethyl siloxane fiber (DVB/CAR-PDMS) was activated for 2 hours at 300°C before use. A 5 mL of various broth obtained was taken and added into a flat bottom headspace vial and sealed with holed polypropylene cap and PTFE/silicone septa. All of the broths were incubated at 50°C in a water bath for 30 mins, and the fiber DVB/CAR-PDMS was exposed to the headspace of the vial for 60 mins at room temperature. The various vapors captured in the headspace were analyzed their volatile compounds using GC-MS. The stepwise procedure of SPME followed by GC-MS measurement is depicted in Figure 1.

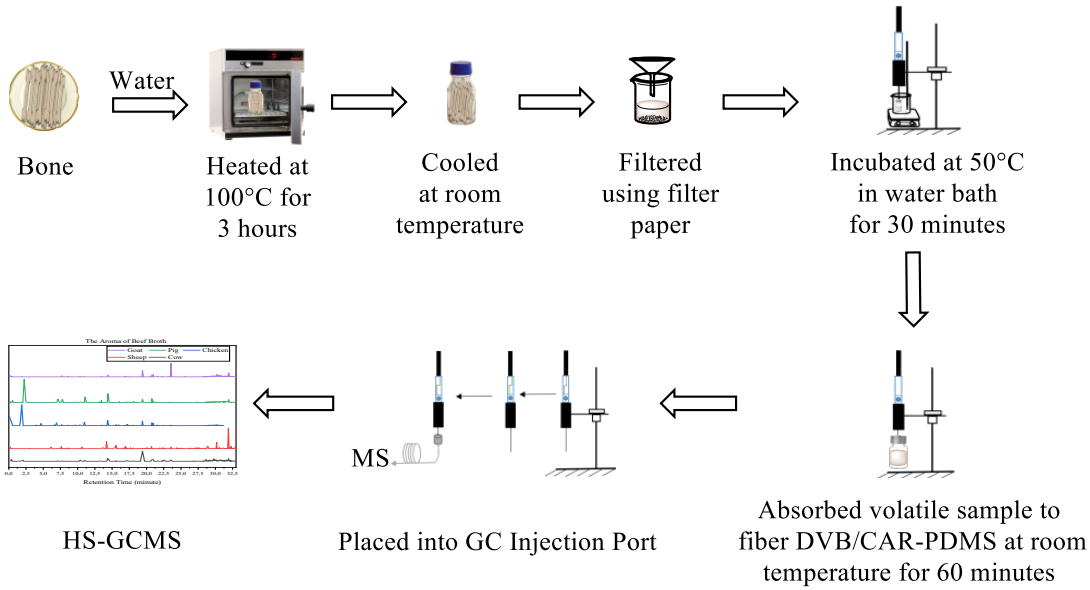

Figure 1. Diagram of analysis with solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS)

Detection of volatile compounds using GCMS Shimadzu QP 2010

The analysis of volatile compounds was performed on a GCMS Shimadzu QP 2010. Thermal desorption of the compounds from the SPME fiber was carried out in the injector port at 230 °C in the split-less mode for 5 min with 1 µL injection. Separations by GC were performed on a polar capillary column Rtx-wax (30 m x 0.25 mm x 0.25 µm). Ultra-high purity helium (99.999%) was used as the carrier gas at a column flow rate of 1 mL/min with a total flow of 10 mL/min. The oven was held for 5 mins at 40 °C, then heated to 100°C at an increasing speed of 5 °C/min, next heated to 240°C at an increasing speed of 10 °C/min and kept constant for 5 mins. The mass spectrometer was operated with an ion-source temperature of 210 °C and interface temperature of 230°C. The mass spectrometer scanned the masses from 40 to 300 m/z. The ion source temperature was at 150°C in electron impact mode at 70 eV. The transfer line temperature was at 230°C. The MS was detected in the 50-450 mass range with a solvent delay of 2.5 mins. Identifcations of unknown compounds were realized by matching GC-MS data and databases (NIST Chemistry WebBook), selecting the components with similarity greater than 60 (maximum 100) and combining them with the retention indices.

RESULT AND DISCUSSION

Determination of broth pH, density, and viscosity

The flavor of broth from different animal profound characteristics, therefore it is interesting to investigate the physical properties which possibly can be used a mean for identification. Flavor is influenced by the quality of the meat and bones, the length of storage, and the type of animals. Broth from meat consists of soluble protein in meat including enzymes and myoglobin, carbohydrates, such as glucose and galactose, polysaccharides, amino acids, lipids, fatty acids, and aromatic compounds which could affect broth pH, viscosity, and density. The results of pH, viscosity and density of all types meat broth is presented in Table 1 showing that all of the meat broth is slightly acidic. The acid compounds found in meat are probably lactic acid formed by glycolysis glycolic acid and succinic acids. Some other acids of the Krebs cycle could present in negligible amounts. Based on Table 1, there is no significant different in pH, viscosity, and density among all types of meat broth, except of the viscosity of chicken

meat broth which showed the highest compared to ruminant broth. This is because chicken meat is softer and thus, it is easier to dissolve during the heating process. The high viscosity of broth is derived from a high concentration of solubilized proteins.

Table 1. The pH, viscosity, and density of different types of meat broth

Broth	pH	Viscosity (mPa.s)	Density (g/ml)
Pork	6.31 ± 0.08	30.5 ± 0.6	1.0028 ± 0.0003
Beef	6.37 ± 0.06	31.8 ± 0.9	1.0033 ± 0.0003
Goat	6.41 ± 0.05	33.5 ± 0.6	1.0029 ± 0.0005
Lamb	6.37 ± 0.04	32.8 ± 0.3	1.0066 ± 0.0004
Chicken	6.35 ± 0.06	42.8 ± 0.3	1.0033 ± 0.0002

Meanwhile, bone contains mineral salts in a considerable amount. Bone also contains collagen which is dissolved in water when heated and could be hydrolyzed to produce amino acids. The measurement results of pH, viscosity, and density of all types of bone broth are depicted in Table 2 revealing that there is almost no significant difference in pH, viscosity, and density among all types of meat broth.

Table 2. The pH, viscosity, and density of different types of bone broth

Broth	pH	Viscosity (mPa.s)	Density (g/ml)
Pork	6.83 ± 0.11	27.8 ± 0.2	0.9984 ± 0.0004
Beef	6.84 ± 0.09	29.5 ± 0.4	1.0027 ± 0.0008
Goat	6.88 ± 0.04	31.5 ± 0.4	1.0001 ± 0.0006
Lamb	6.89 ± 0.07	30.8 ± 0.7	1.0009 ± 0.0002
Chicken	6.87 ± 0.06	32.0 ± 0.4	1.0003 ± 0.0002

It can be seen from Table 2 that the pH value of bone broth was close to neutral. The pH of bone broth tends to be higher than that of meat broth because not only there is the amino acids content, but also there are dissolved alkaline minerals released from the bones. The ingredients contribute to the viscosity and density of bone broth are marrow and collagen.

Comparison of these physical properties between meat and bone broth (Table 1 and Table 2) showing that pH values of all meat broth have a lower trend than those of bone broth; however, viscosity and density of meat broth are slightly higher than those of the bone broth. This is because, physically, the meat has a softer texture than the bone and thus easily crumble and dissolve in water. Notably, the physical properties of meat and bone broth, i.e., pH, viscosity, and density, cannot be used to distinguish the type of broth produced from beef, pork, goat, lamb, and chicken.

Determination of the predominant volatile compound of meat broth

This research was conducted to identify volatile compounds using GCMS QP-2010 with SPME fiber adsorption method 50/30µm DVB/CAR/PDMS (divinylbenzene/ carboxen/polydimethylsiloxane. DVB/CAR/PDMS fiber can extract the most effective volatile compounds compared to other types of fiber such as PDMS, PA, PDMS/DVB, and PDMS/CAR [19]. The combination of coating materials in DVB/CAR/PDMS can extract volatile compounds through physical adsorption mechanisms. The DVB/PDMS layer is a coating material that has medium to large pores (meso-macropores) which function to absorb

large compound particles, while the smaller compound particles are absorbed by the CAR/PDMS layer which has small pores (micropores). The CAR/PDMS layer is effective for absorbing volatile compounds with low boiling points, while the DVB/PDMS layer is effective for volatile compounds with high boiling points [9]. Therefore, PDMS/DVB/CAR fiber is a fiber with a proper combination of coating materials and produces a wide range of compounds that are adsorbed. PDMS/DVB/CAR fiber has been widely used in aroma analysis in meat products because it can extract organic substances from short carbon chains to long carbon chains from C3 to C20.

The chromatogram of volatile compounds of meat broth is depicted in Figure 2 and they are composed of hydrocarbons, alcohols, aldehydes, acids, esters, ketones, furans, and S-containing compounds. The profile of chromatogram showed characteristics for each type of meat broth in term of the appearance of peaks and the intensity of peaks. This finding, chromatograms from volatile compound of meat broth, can be proposed as a mean for halal and non halal identification. The predominant volatile compounds from each meat broth are listed from the greatest abundance to the smallest in Table 3. The aldehyde group plays an important role in the aroma of meat. It is not only present in large numbers, but it is also present in a high percentage area. In this study, the aldehydes that always appeared in all broth samples were aliphatic aldehyde groups, including hexanal, heptanal, octanal, nonanal, and decanal. Hexanal is the compound that appears with the highest percentage in each sample. The volatile compounds of the aldehyde group are formed by the degradation of linolenic acid.

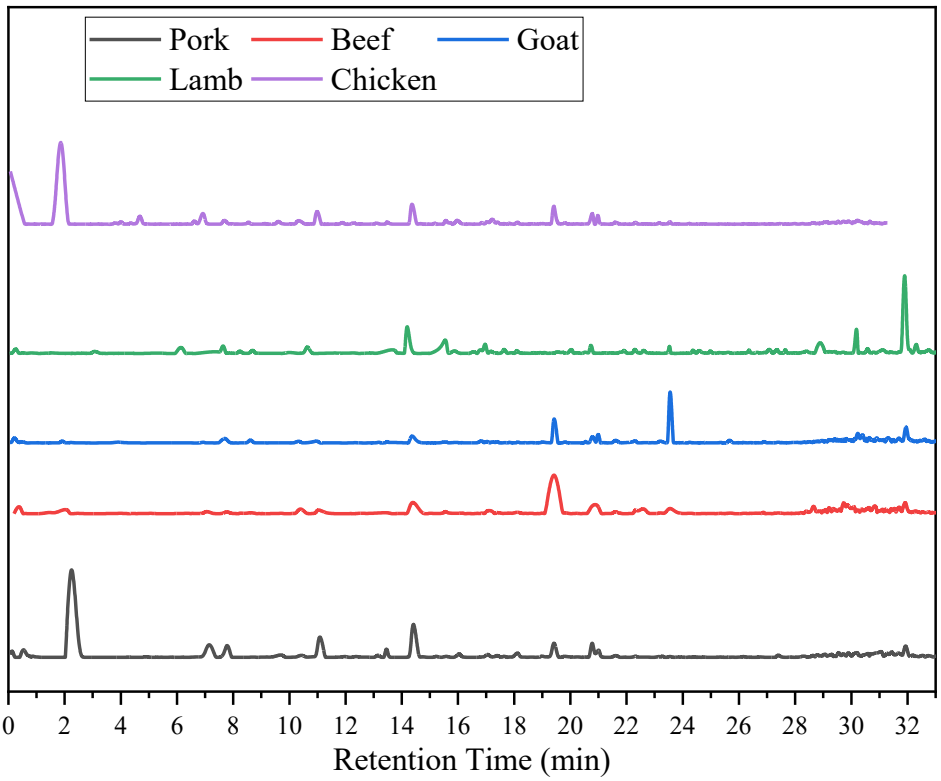

Figure 2. Chromatogram of volatile compounds from meat broth

Volatile compounds from meat pork based on Table 3 consist of compounds from the aldehyde group, namely hexanal, nonanal, octanal, heptanal, decanal and 2-octenal, 2-nonenal, where aldehyde compounds are formed due to oxidation of carbon double bonds from saturated and unsaturated fatty acids. Compounds from the alcohol group were also identified such as 1-

octanol, 1-pentanol, 1-heptanol, 1-hexanol, and 1-dodecanol. Alcohol groups occur during processing due to fat oxidation, fatty acids, and amino acid degradation [3]. The results also found some aromatic compounds such as naphthalene and methyl-d3-1-dideuterio-2-propenyl ether.

The identification of the main volatile compounds of beef broth was listed in Table 3 while the five most dominant compounds were isomenthol, nonanal, oxime-methoxy-phenyl, naphthalene and methyl benzene. Isomenthol is only found in boiled beef and not in raw beef [8]. The composition of volatile compounds in beef and pork broth is generally dominated by aldehyde group compounds. The aldehyde groups compounds such as hexanal, octanal, and nonanal have high concentrations in pork broth samples. Nonanal and octanal are compounds resulting from the oxidation of oleic acid, while hexanal are volatile compounds produced from the oxidation reaction of linoleic acid. These two fatty acids are the most common unsaturated fatty acids found in pork. By using the SPME method, the most extracted compound in pork broth is aldehyde [3].

Broth from goat meat contains 22 most abundant volatile compounds as listed in Table 3. There are five aldehyde compounds identified in goat meat, namely nonanal, hexanal, octanal, decanal, and undecanal. There are also alcohols, namely 1-dodecanol and 2-ethyl-1-hexanol. The identified hydrocarbon compounds are methoxy phenyl oxime, 1-(2 Aminophenyl) ethanone oxime, naphthalene, methyl benzene, and 1,2-dichlorobenzene.
The compound of the identified ester is bis(2-ethylhexyl) hexanoic ester. The compound 1-dodecanol is the most abundant in goat meat and bones, this allows the compound to provide a strong distributor with a high concentration of goat aroma. The results of lamb broth identified using GC-MS showed 25 of the most abundant compounds. The five most dominant compounds with high abundance were hexanedioic acid, bis(2-ethylhexyl) ester, nonanal, 1,2-diphenoxyethane, octanoate ethyl ester acid, and tetracosamethyl cyclododecyloxane. Volatile compounds in pork and lamb broth are generally dominated by aldehyde group compounds. The aldehyde group compounds include n-hexanal, n-octanal, n-nonanal, and n-decanal.

The composition of volatile compounds in chicken broth was close to those from pork broth. The aldehyde group compounds in chicken broth have the highest abundance and include hexanal, nonanal, octanal, and heptanal. The alcohol groups that appeared in all broths with a high percentage area. 1-octen-3-ol, 1-dodecanol 1-pentanol, and 1-heptanol were the highest percent area in chicken broth. The main origin of the formation of alcohol groups in volatile compounds is oxidative fat decomposition reactions. Siloxane groups also appeared in all broth samples included in chicken broth.

Determination of the predominant volatile compound of bone broth

For the bone broth, the chromatogram profile resulted from GC-MS on samples of pork bone broth showed characteristics for each type of animals (Figure 3) with most abundant compounds as shown in Table 4. Each chromatogram, in general, mostly contained peaks which fall at similar retention time; however, the intensity of signal of the corresponding peak is different from one type of animal to another. Therefore, the profile of the chromatogram is feasible to be used to distinguish the origin of animals.

Based on the mass spectra, the identified volatile compounds in bone broth consisted of seven compounds of the aldehyde group, such as hexanal, nonanal, octanal, heptanal, 2-heptenal, 2,4-decadienal and 2-octenal. The alcohol group were also detected including 1-pentanol, 1-octen-3-ol and 1-octanol. Other compounds detected were aromatic and aliphatic hydrocarbon groups, such as methyl benzene, 1,3-dichlorobenzene, naphthalene, and oxime

methoxy phenyl and 1 ketone group of 2,3-octadione. Aldehyde compounds give taste to meat because it has a low odor threshold and gives a specific aroma.

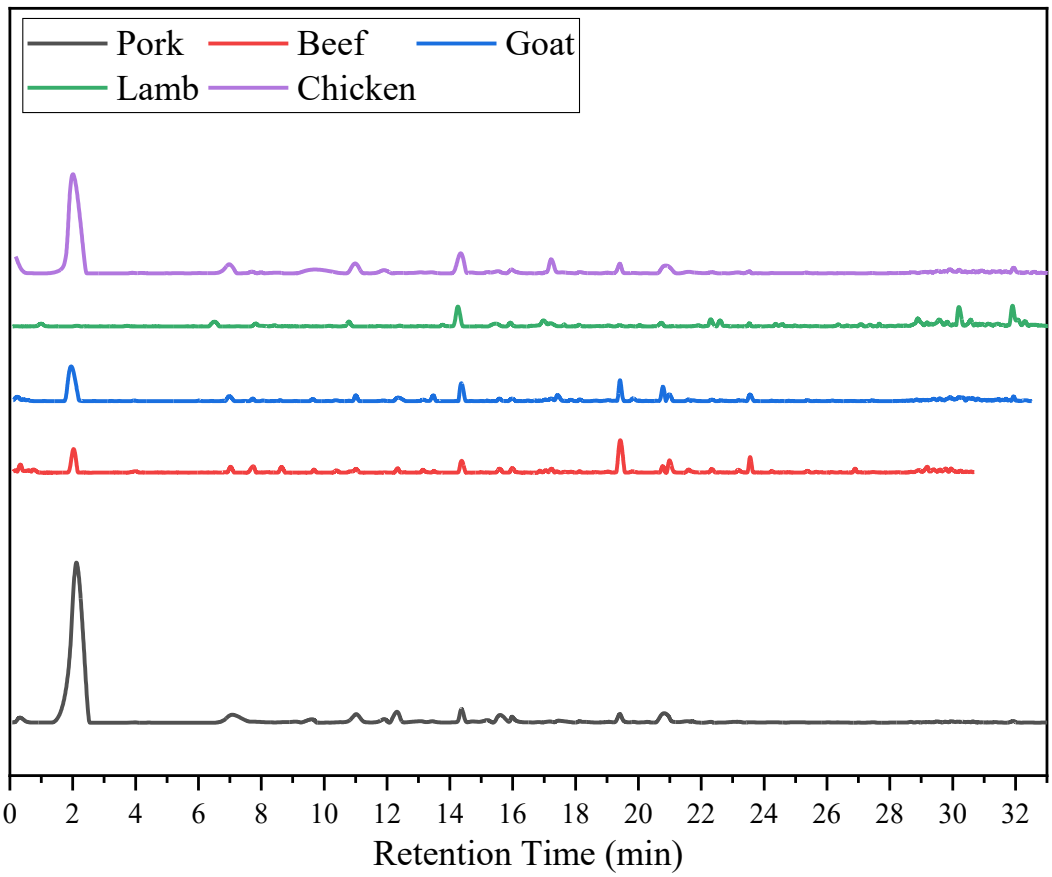

Figure 3. Chromatogram of volatile compounds from bone broth

Table 3. The predominant characteristic volatile compound of meat broth

No	Pork	Beef	Goat	Lamb	Chicken
1	Hexanal	Isomenthol	1-Dodecanol	Hexanedioic acid, bis(2-ethylhexyl) ester	Hexanal
2	Nonanal	Nonanal	Methyl-d3 1-Dideuterio-2-propenyl Ether	Nonanal	Methyl Benzene
3	Octanal	Oxime-, methoxy-phenyl-	Hexanedioic acid, bis(2-ethylhexyl) ester	1,2-Bis(2-phenoxyethoxy)ethane	Nonanal
4	Naphthalene	Naphthalene	Oxime-, methoxy-phenyl-	Ethyl octanoate	Methyl-d3 1-Dideuterio-2-propenyl ether
5	Methyl-d3 1-Dideuterio-2-propenyl ether	Methyl Benzene	Nonanal	Decanal	Octanal
6	Decamethylcyclopentasiloxane	1-Dodecanol	Naphthalene	1,2-Bis(methoxymethyl)benzene	Naphthalene
7	Heptanal	Hexanedioic acid, bis(2-ethylhexyl) ester	Decamethylcyclopentasiloxane	Azulene	Oxime-, methoxy-phenyl-
8	Dodecamethylcyclohexasiloxane	[4,4-dimethyl-3-(2-methylpropanoyloxy)pentan-2-yl] 2-methylpropanoate	1-(2-Aminophenyl)ethanone oxime	Decamethylcyclopentasiloxane	n-heptanal
9	Dioctyl hexanedioate	3-Hydroxy-2-butanone	Bis(2-ethylhexyl)	Heptanal	Xylene
10	2-Undecenal	Hexanal	Methyl Benzene	Octanal	N-benzylidene-dimethylammonium chloride
11	Oxime-, methoxy-phenyl-	Neryl acetone	1-Dodecanol	Cyclododecane	1-Octen-3-ol
12	2-Decenal	Octanal	Hexamethylcyclotrisiloxane	Tetracosamethylcyclododecasiloxane	Decamethylcyclopentasiloxane
13	1-Octanol	Decanal	2-ethyl-1-Hexanol	Tetradecamethylcycloheptasiloxane	Hexanedioic acid, bis(2-ethylhexyl) ester
14	Methyl Benzene	Decamethylcyclopentasiloxane	Octamethylcyclotetrasiloxane,	2-ethyl-1-Hexanol	1-Decene
15	Chloroform	Urs-12-en-28-ol	Octanal	6,10-Dimethyl-5,9-undecadien-2-one	1,4-Dichlorobenzene
16	1-Heptanol	1,4-Dichlorobenzene	3-Hydroxy-2-butanone	alpha-Hexylcinnamaldehyde	3-Hydroxy-2-butanone
17	Decanal	Benzylidene dimethylammonium chloride	Ethyl 3-methylbutanoate	Hexanal	3-Dodecen-1-al
18	1-Hexanol	Hexamethylcyclotrisiloxane	Diethyl 1,2-hydrazinedicarboxylate	Octadecamethylcyclononasiloxane	1,2-Dimethylbenzene
19	1-Pentanol	Ethyl 3-hydroxybutyrate	Decanal	1,2-Dichlorobenzene	1-Dodecanol
20	Diethyl phthalate	Heptanal	Hexanal	Lily aldehyde	Decanal
21	3-Hydroxy-2-butanone	L-Isoleucine	Undecanal	Dodecamethylcyclohexasiloxane	1-Pentanol
22	Tetradecamethylcycloheptasiloxane	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	Dodecamethylcyclohexasiloxane	Ethyl hexanoate	
23	1-Octen-3-ol			1-Octanol	
24	Hexamethylcyclotrisiloxane			Hexadecamethylcyclooctasiloxane	
				2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	

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Table 4. The predominant volatile compound of bone broth

No	Pork	Beef	Goat	Lamb	Chicken
1	Hexanal	Methyl-d3 1-Dideuterio-2-propenyl Ether	Hexanal	Nonanal	Hexanal
2	Nonanal	Hexanal	Methyl-d3 1-Dideuterio-2-propenyl Ether	Hexanedioic acid, bis(2-ethylhexyl) ester	Nonanal
3	2,3-Octanedione	1-Dodecanol	Nonanal	1,2-Bis(2-phenoxyethoxy)ethane	Benzaldehyde
4	Methyl-d3 1-Dideuterio-2-propenyl Ether	Nonanal	Naphthalene	Neryl Acetone	Octanal
5	Naphthalene	Oxime-, methoxy-phenyl-	Tetradecamethylcycloheptasiloxane	Decanal	Methyl Benzene
6	Octanal	Naphthalene	1-Dodecanol	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	Methyl-d3 1-Dideuterio-2-propenyl Ether
7	Heptanal	Decamethylcyclopentasiloxane	Oxime-, methoxy-phenyl-	Octanal	Heptanal
8	1,3-Dichlorobenzene	Di(2-ethylhexyl)adipate	Octanal	Heptanal	Naphthalene
9	1-Octen-3-ol	1-(2-Aminophenyl)ethanone oxime	Dodecamethylcyclohexasiloxane	Tetracosamethylcyclododecasiloxane	Oxime-, methoxy-phenyl-
10	Oxime-, methoxy-phenyl-	Heptanal	Heptanal	1-Octen-3-ol	1-Pentanol
11	Methyl Benzene	1-Octen-3-ol	2,3-Octanedione	Azulene	1-Octen-3-ol
12	2-Heptenal	2,3-Octanedione	Hexanedioic acid, bis(2-ethylhexyl) ester	1-Dodecanol	2-Heptenal
13	1-Pentanol	P-Octyl-oxy-benzoic acid	Hexadecamethylcyclooctasiloxane	Lily Aldehyde	Dioctyl hexanedioate
14	2 Octenal	Octamethylcyclotetrasiloxane	Decamethylcyclopentasiloxane	Octadecamethylcyclononasiloxane	1-Dodecanol
15	Tetradecamethylcycloheptasiloxane	N-benzylidene-dimethylammonium chloride	1-Octen-3-ol	N-benzylidene-dimethylammonium chloride	Decamethylcyclopentasiloxane
16	2,4-Decadienal	Octanal	N-benzylidene-dimethylammonium chloride	1-(4-Isopropylphenyl)-2-Methylpropyl Acetate	1,4-Dichlorobenzene
17	1-Octanol	Methyl Benzene	Methyl Benzene	1,4-Dichlorobenzene	Hexamethylcyclotrisiloxane
18	1-Heptanol	1,2-Dichlorobenzene	1-Pentanol	Hexanal	1-Heptanol
19	Hexamethylcyclotrisiloxane	Hexamethylcyclotrisiloxane	1-Octanol	Tetradecamethylcycloheptasiloxane	1-Octanol
20	Dioctyl hexanedioate	1-Pentanol	1,4-Dichlorobenzene	3-Ethylidene-1-methyl-1,4-cycloheptadiene	2-Octenal
21	Dodecamethylcyclohexasiloxane	1-Hexanol	Hexamethylcyclotrisiloxane	Hexamethyl-pyranoindane	Decanal
22	1-Hexanol	Decanal	Decanal	Decamethylcyclopentasiloxane	Dodecamethylcyclohexasiloxane
23	2-Decenal	2-Ethylhexanol	1-Heptanol	1-Octanol	3-Ethyl-2-Methyl-1,3-Hexadiene
24	3-Ethyl-2-Methyl-1,3-Hexadiene	3-Hydroxy-2-butanone	2,5-bis[(trimethylsilyl)oxy]-Benzaldehyde	Hexadecamethylcyclooctasiloxane	Octamethylcyclotetrasiloxane
25	Benzaldehyde	Diethyleneglycol mono-n-propyl ether	Octamethylcyclotetrasiloxane	Hexamethyl-pyranoindane	3-Methyl-1-butanol
26	2-(1,1-dimethylethyl)-Cyclohexanol	1-Heptanol	1-(2-Aminophenyl)ethanone oxime	alpha-hexylcinnamaldehyde	Hexyl formate
27	Trans-nona-2,4-Dienal	1-Octanol	1-Hexanol	2-Octen-1-ol	1-Decene
28	Hexyl-oxirane		Tris(trimethylsiloxy)arsine		14-Octadecenal

The results of GC-MS on samples of pork bone broth obtained a chromatogram 28 most abundant compounds shown in Table 4. There are seven compounds of the aldehyde group formed in pork bones, namely hexanal, nonanal, octanal, heptanal, 2-heptenal, 2,4-decadienal and 2-octenal. The compounds from the alcohol group detected were three compounds, namely 1-pentanol, 1-octen-3-ol and 1-octanol. Compounds from the aromatic and aliphatic hydrocarbon groups are methyl benzene and 1,3-dichlorobenzene, naphthalene, oxime methoxy phenyl. Compounds from the ketone group were detected in pork bones, namely 2,3-octadione. Aldehyde compounds give taste to meat because it has a low odor threshold, so it gives a specific aroma.

The predominant volatile compounds present in the beef bone broth are chosen from the main peaks that have the potential to give a distinctive aroma. Methyl-d3-1-dideuterio-2-propenyl ether, hexanal, 1-dodecanol nonanal, Oxime-, methoxy-phenyl- and naphthalene were the compounds with the highest abundances. Aldehyde group compounds such as hexanal, heptanal, nonanal, and octanal have an important role in the aroma of pork bone broth and beef ribs. This means that the composition of the beef and pork bone broth samples is dominated by aldehyde group compounds, the difference between the two is the percentage area of the aldehyde group compound where the pork bone sample is larger than that of the beef bone.

The 28 compounds being the most abundant in goat bone broth samples. Based on Table 4, there are identified aldehyde compounds such as hexanal, heptanal, decanal, octanal, and nonanal. The compounds from the alcohol group are 1-pentanol, 2-propanol, 1-heptanol, 1-octanol, and 1-dodecanol. Hexanal is the most abundant in goat bones and the other compounds are methyl-d3 1-dideuterio-2-propenyl ether, hexanal, 1-dodecanol nonanal, oxime-, methoxy-phenyl- and naphthalene. Volatile compounds from lamb bone broth were found 27. The highest percent area that had the potential to give a distinctive aroma to the sample were the five most dominant compounds and have a high abundance in the lamb bone broth samples so that they have the potential to be typical compounds are nonanal, hexanoic acid bis (2-ethylhexyl) ester, 1,2-bis(2-phenoxyethoxy) ethane, neryl acetone, and decanal.

The 28 volatile compounds in chicken bone broth give it a distinctive aroma. Aldehydes are the main volatile components of chicken bone broth. Hexanal compounds have the highest percentage of area in chicken bone broth. Nonanal, octanal, and heptanal are also present in a high percentage. 1-pentanol has the highest percentage of alcohol group in chicken bone broth. An ether group was also found in this sample namely methyl-d3 1-dideuterio-2-propenyl ether. From the chromatogram also appeared organosilicon compounds such as decamethyl-cyclopentasiloxane, hexamethylcyclotrisiloxane, dodecamethylcyclo-hexasiloxane and octamethylcyclotetrasiloxane.

Determination of specific compounds in pork broth

The aroma that appears in all broth samples is mostly aldehyde compounds. Hexanal is described as smelling grass and fat; octanal has a fatty and sour aroma, while nonanal has a roasted, fatty, and sweet aroma [4]. Nonanal and octanal are compounds resulting from the oxidation of oleic acid, while hexanal are volatile compounds produced from the oxidation reaction of linoleic acid. From 5 samples of meat broth: pork, beef, goat, lamb, and chicken, the compounds that always appear with different percentages are methyl benzene, nonanal, and naphthalene. Pork broth had the highest levels of nonanal compounds compared to the other four broths. The volatile compounds which always present in bone broth samples are also

aldehyde group compounds such as hexanal and nonanal. From 5 compounds of bone broth; pork, beef, chicken, and goat, compounds that always appear with different percentages are hexanal, nonanal, methyl-d3 1-dideuterio-2-propenyl ether, and naphthalene. All bone broths had the highest levels of hexanal, but pork bone broth had the highest levels of hexanal compounds compared to the other three broths. Specific compounds are compounds that only appear in one broth and have the potential as a marker to identify what animal broth is made of. The predominant compound contained in one type of broth is compared with the compounds found in other broths to obtain the specific compound.

Specific compounds are compounds that only appear in one broth and have the potential as a marker to identify the typical animal broth. Thus, to obtain the specific compound, the predominant compound contained in one type of broth is compared with the compounds found in other broth. Volatile compounds with the highest abundance in each broth are the main constituents of the broth aroma. The specific compounds of the broth are determined by selecting each compound in one type of broth that is not found in other broth. The possible volatile marker for pork broth was determined when the compound was only found in one kind of broth and not found in other broths as listed in Table 5.

Table 5. The specific volatile compound of broth pork

No	Meat broth	Bone broth
1	Dioctyl hexanedioate	1,3-Dichlorobenzene
2	(E)-2-Undecenal	2-Octenal
3	2-Decenal	2,4-Decadienal
4	1-Hexanol	2-Decenal
5	1-Pentanol	3-Ethyl-2-methyl-1,3-hexadiene
6	Diethyl phthalate	2-(1,1-dimethylethyl)-cyclohexanol
7	Tetradecamethylcycloheptasiloxane	Trans-nona-2,4-dienal
8	-	Hexyl oxirane

Volatile compounds with the highest abundance in each broth are the main constituents of the broth aroma. The specific compounds of the broth are determined by selecting each compound in one type of broth that is not found in other broths. The possible volatile marker was determined when the compound was only found in one kind of broth and not found in other broths as listed in table 5. The specific compounds as dioctyl hexanedioate, (E)-2-undecenal, 2-decenal, 1-hexanol, 1-pentanol, diethyl phthalate and tetradecamethyl-cycloheptasiloxane were only found in pork meat broth, while 1,3-dichlorobenzene, 2-octenal, 2,4-decadienal, 2-decenal, 3-ethyl-2-methyl-1,3-hexadiene, 2-(1,1-dimethylethyl) cyclo-hexanol, trans-nona-2,4-dienal and hexyl oxirane were only found in pork bone.

The other broth also five largest specific compounds such as isomenthol, [4,4-dimethyl-3-(2-methylpropanoyloxy) pentan-2-yl]2-methylpropanoate, neryl acetone, urs-12-en-28-ol, and benzylidene dimethylammonium chloride were only found in beef meat while di(2-ethylhexyl) adipate, 1-(2-aminophenyl) ethanone oxime, p-octyl-oxy-benzoic acid, 1,2-dichlorobenzene, and 2-ethylhexanol were only found in beef bone. The five largest specific compounds such as 1-(2-aminophenyl) ethanone oxime, octamethylcyclotetrasiloxane, ethyl 3-methylbutanoate, diethyl 1,2-hydrazinedicarboxylate and undecanal were only found in goat meat broth, while hexanedioic acid, bis(2-ethylhexyl) ester, N-benzylidene-dimethyl-ammonium chloride, 2,5-bis[(trimethylsilyl)oxy]-benzaldehyde, 1-(2-aminophenyl) ethanone oxime and tris-(trimethylsiloxy) arsine were only found in goat bone. The five largest

specific compounds such as 1,2-bis(2-phenoxyethoxy) ethane, ethyl octanoate,1,2-Bis(methoxymethyl)benzene, azulene and cyclododecane were only found in lamb meat broth, while hexanedioic acid bis(2-ethylhexyl) ester, 1,2-bis(2-phenoxyethoxy) ethane, neryl acetone, azulene and lily aldehyde were only found in lamb bone. The five largest specific compounds such as xylene, n-benzylidene-dimethylammonium chloride, 1-decene, 3-dodecen-1-al, and, 1,2-dimethyl benzene were only found in chicken meat broth, while 3-ethyl-2-methyl-1,3-hexadiene, 3-methyl-1-butanol, hexyl formate, 1-decene, and 14-octadecenal were only found in chicken bone. The selection of specific compounds in each broth can be potentially as a marker compound to identify the type of broth and further allow it to be used for halal detection.

CONCLUSION

The physical properties as well as volatile compounds using SPME-GC-MS in meat and bone broth from pork, beef, goat, lamb, and chicken has been investigated as one of many means for identification of halal and non halal meat. The pH, viscosity, and density of meat and bone broth gave results to similar value to all types of animals and cannot be used for halal and non halal identification. Successful results for the identification were achieved through the profile chromatogram of volatile compounds from both meat and bone broth which shows characteristic to each type of animals, and therefore it can be used to distinguish pork from beef, goat, lamb, and chicken. This research also founds 7 predominant volatile compounds as a marker for pork meat and other 8 specific compounds marker for pork bone. These findings can potentially be developed as a method to identify the type of broth and further allow it to be used for halal and non halal detection.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Diana Candra Dewi conducted the experiment and write draft of the manuscript, Hermin Sulistyarti and Chanif Mahdi wrote and revised the manuscript, and Aulani'am conducted proof reading. All authors agreed to the final version of this manuscript.

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