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Physicochemical characteristics and SPME-GC-MS based volatilomics for discrimination of beef and pork patties

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Abstract

Beef is the preferred main ingredient for patties due to its high nutritional value; however, it is also a costly commodity. As a result, beef is often adulterated or partially substituted with pork, which can raise ethical and religious concerns. This study aimed to investigate the physicochemical and volatilome characteristics of patties made from beef, pork, and their mixtures at varying ratios, both with and without added seasonings (salt, pepper, and garlic). Physicochemical properties—including proximate composition, water-holding capacity, cooking loss, texture, and color—were analyzed to assess how pork and seasoning additions influence the characteristics of beef patties. Volatilome analysis was performed using solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC-MS). Data were analyzed using principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) to identify volatile compounds affected by the inclusion of pork. The results showed that adding 25% pork and seasoning altered the physicochemical properties of beef patties. These additions also affected the volatilome profiles. Key volatile marker compounds identified included Dimethyl disulfide (beef patties), Naphthalene (mixed patties), and Hexanal (pork patties) in seasoned samples. For unseasoned samples, potential markers were 3,7,11- Trimethyl-1-dodecanol, Hexadecane, and Nonanal for beef patties; Naphthalene, Octanal, and Heptanal for mixed patties; and Hexanal, (E)-2-Octenal, (E)-2-Heptenal, and 2-Pentylfuran for pork patties. These findings demonstrate that both physicochemical and volatilomic analyses are effective tools for distinguishing between patties made from beef, pork, and their mixtures. Future studies should evaluate whether these compositional changes influence the sensory properties of the patties. As a chemical validation, quantification of the identified markers using reference compounds is also required.

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1. Introduction

While having a high nutritional value, beef is an expensive commodity. As a result, beef as the primary constituent of meat products is frequently falsified and replaced with cheaper meat, such as pork, buffalo, and horse (1,2). In Indonesia, pork is frequently adulterated with beef. An example of this case was recently reported in Indonesia. The study showed that 22 among 36 commercial meatball samples in Boyolali Regency, Central Java, were detected to contain pork DNA using PCR (3).This is greatly worrying for some ethical and religious concerns. Pork and wild boar are forbidden for Muslims to consume because they are considered non-halal (forbidden) animals. Halal food authentication techniques have been developed over the years, including studies on meat adulteration detections and quality checking, such as studies using physicochemical techniques, polymerase chain reaction techniques, hyperspectral imaging techniques, multispectral imaging systems, and many more (4–6). These techniques have both advantages and disadvantages in halal food authentication, especially concerning the complexity of food ingredients. They need rigorous sample preparation and technical expertise, which makes them less than ideal for routine analysis (7).Therefore, the topic of this study is related to the innovation of halal detection methods that are easier and more suitable for complex food ingredients.

Physicochemical analysis is commonly used to identify the chemical and physical properties of food, owing to its low cost and ease of application. The volatile profile of meat is a complex combination of hundreds of molecules that contribute to its aroma. These volatile compositions can be affected by various conditions, such as feed, breed, storage, species, and cooking methods (8). Although raw meat has fewer volatiles in common; thus, it has a lesser aroma, previous studies have reported that raw beef or pork stored at different periods and storage conditions have different volatile compositions (9,10). Previous studies have also shown that meat from different species, such as beef and pork, has a distinct volatile profile, either in uncooked (11,12) or cooked forms (7,13). Several reactions occur during meat processing, such as the Maillard reaction, lipid oxidation, and Thiamine degradation, as well as interactions between the reaction intermediates and end products, which result in the formation of new volatile compounds. These volatile compounds play an important role in the formation of a typical meat flavor (8).

Solid-phase microextraction (SPME) combined with GC-MS is the most commonly used technique for analyzing volatile organic metabolites (VOMs). This technique is highly discriminating, quick, and economical, and it performs well owing to the accessibility of the extraction materials and fundamental equilibration mechanisms (14). Nevertheless, because of SPME fiber efficiency loss after numerous extractions, the SPME-based volatilomics technique has poor repeatability. Despite the fact that various normalization approaches have been created, their effectiveness varies greatly and is heavily dependent on the type of data being studied (15). However, the sensitivity of MS instruments is presently used to overcome this shortcoming (16). As it is, sample drawings are not necessary, and less pre-analysis processing is required when biological systems are extracted directly. Given their protection from enzymatic activity and the lack of chemical modification during ex vivo analysis, the captured metabolites offer a more precise depiction of the system under investigation, encompassing any unstable molecules that may have been present during sampling. Previous studies have successfully used this technique to differentiate meat of different species have been published. Pranata and co-workers successfully differentiated chicken, wild boar, and beef meatballs using SPME-GC-MS and multivariate data analysis (7),

where β-Cymene, 3-Methyl-butanal, and 2-Pentanol were discovered as discriminating volatiles in chicken meatballs. Beef meatballs were distinguished by their high levels of 5-Ethyl-m-xylene, Benzaldehyde, and 3-Ethyl-2-methyl-1,3-hexadiene. The pure wild boar meatballs had a higher concentrations of pentanal, 2,6-Dimethylcyclohexanone, 1-Undecanol, Cyclobutanol and 2,4,5-Thiazole. The same technique was also used to differentiate between beef, wild boar, and rat meatballs. Three of the strongest markers in beef, rat, and wild boar meatballs were identified as (Z)-2-Amino-5-methyl-benzoic acid, 2-Heptenal, and Cyclobutanol, respectively (17).

Previous examples of volatile-based studies have mostly used meatball sampling. This is because meatballs are among the most popular street foods in Indonesia. However, Indonesian consumers also favor patties, as seen by the large number of eateries and food booths that provide them. Similar to meatballs, beef patties can also be adulterated by non-halal meat such as pork. Therefore, this study aims to examine key volatile compounds distinguishing beef, pork, and mixed-meat patties at varying ratios. The differences in physicochemical characteristics that include water-holding capacity, cooking loss, texture, color, and proximate composition, were also investigated. The effect of additional seasoning on the abovementioned characteristics of the patty was also studied. The volatilome profile was analyzed using SPME coupled to GC-MS. The data were analyzed using multivariate data analysis techniques, Principal Component Analysis (PCA) and Orthogonal Projection to the Least Square Analysis (OPLS-DA). Volatile marker selection was based on the value of the correlation coefficient (positive) and high variable importance to the projection (> 1).

2. Materials and Methods

2.1. Materials

Minced beef and pork tenderloins were obtained from the Cijantung Jaya Market in East Jakarta (Indonesia) as the key ingredients for this experiment. Other ingredients include fresh garlic, salt (Dolphin), and white pepper powder. Alkane standards C8-C40 (Polyscience, Illinois, USA), NaCl PA and methanol PA (Merck, Darmstadt, Germany), and distilled water are analytical chemicals that are required. All chemicals used were of analytical grade.

2.2. Methods

2.2.1. Patty Preparations

The garlic cloves were then mashed using a blender. All the necessary materials were weighed as shown in Table 1. The gloved hands were used to mix the ingredients until they were homogenous. A plastic disk-shaped mold with a diameter of 70 mm was used to form up to 50 g of sample. Each sample was a thickness of 10 mm. As much as 50 g of margarine was melted in a double-sided Teflon pan. The sample was roasted in the Teflon pan for approximately 4 minutes on one side and 3 minutes on the other side. The pan was then washed and dried after every use.

Table 1. Formulation of patty's ingredient composition.

Sample No.	Minced beef (g/100g meat)	Minced pork (g/100g meat)	Salt (g/100g meat)	Pepper (g/100g meat)	Garlic (g/100g meat)	Sample Code
1	50.00	0.00	0.00	0.00	0.00	100_0
2	37.50	12.50	0.00	0.00	0.00	75_25
3	25.00	25.00	0.00	0.00	0.00	50_50
4	12.50	37.50	0.00	0.00	0.00	25_75
5	0.00	50.00	0.00	0.00	0.00	0_100
6	50.00	0.00	0.50	0.50	1.25	B100_0
7	37.50	12.50	0.50	0.50	1.25	B75_25
8	25.00	25.00	0.50	0.50	1.25	B50_50
9	12.50	37.50	0.50	0.50	1.25	B25_75
10	0.00	50.00	0.50	0.50	1.25	B0_100

2.2.2. Proximate Analysis

Proximate analyses of water, ash, protein, and fat contents were performed according to the Association of Official Agricultural Chemists (AOAC) guidelines (18).

2.2.3. Color Analysis

A chromameter was used to analyze the color of the patties (Konica Minolta Chromameter CR-400), which is based on the color difference of the sample surface (19). The chromameter was calibrated using a proper white color standard in the instrument. Patty samples were placed on white paper. The results were expressed as L* (lightness), C* (chroma/vividness), and h (hue) values.

2.2.4. Water Holding Capacity Analysis

A meat chopper was used to mince the sample. A 5-gram sample and 7.5 mL of 0.6 M NaCl solution were vortexed for 1 minute in a 15 mL centrifuge tube. After refrigeration for 15 minutes at 4 °C, the tubes were centrifuged at 3000 rpm for 30 minutes at 4 °C (20,21).

$$WHC(\%) = 100\ x\ \frac{(W_{pellet} - W_{raw})(g)}{W_{raw}\ (g)} \quad (1)$$

W_{raw} is the initial weight (g), and W_{pellet} is the sample weight after centrifugation (g).

2.2.5. Cooking Loss Analysis

The samples were sealed in plastic bags and cooked in at 80°C water bath for 30 minutes. The samples were then tempered at room temperature for 30 minutes (22).

$$Cooking\ Loss(\%) = 100\ x\ \frac{(W_1 - W_2)(g)}{W_1\ (g)} \quad (2)$$

W_1 is the sample weight before cooking (g), and W_2 is the weight after cooking (g).

2.2.6. Texture Profile Analysis

A cylindrical probe with a diameter of 20 mm is used. The sample was pushed down by the probe at constant speeds of 3.0 $mm.s^{-1}$ (pre-test), 1.0 $mm.s^{-1}$ (test), and 3.0 $mm.s^{-1}$ (post-test). The probe was pushed lower than 75% of the sample thickness, returned to the initial point of contact with the sample, and halted for a predefined amount of time (2 s) before the second compression cycle began. The resistance of the sample was measured every 0.01 seconds during the test and plotted in a force-time (grams-seconds) plot. All tests used a constant compression speed of 1 $mm.s^{-1}$, and the areas under the force-time curve were exactly proportional to the work done by the probe on the downstroke and the sample on the upstroke. A force-time plot was used to read and measure the texture profile properties (hardness, springiness, cohesiveness, and chewiness) (23).

2.2.7. Volatiles Compound Extraction using SPME

The SPME fibers (DVB/Car/PDMS coatings 50-30 m Supelco (Sigma Aldrich, Bellefonte, USA)) were cleaned by heating the fiber in the GC-MS injection port at 250 ℃ for 5 minutes. The extraction procedure was completed by placing the SPME fiber in a vial for 90 minutes at 80 ℃ for 8 g of the minced sample. GC-MS analysis was performed by inserting SPME fibers containing volatile components into the GC-MS injection port. At an injector temperature of 175 ℃, sample injection was carried out in splitless mode. A GC Capillary Column (Rtx-5MS 30 m, 0.25 mm ID, 0.25 µm) was used. The oven temperature started out at 33 ℃ for 5 minutes before increased at a rate of 10 ℃/min until it reached 200 ℃, where it was maintained for 5 minutes. The transfer line temperature was set at 200 ℃. The temperatures of the MS quadrupole and ion source were 150 and 230 ℃, respectively. For the calculation of the linear retention indices (LRI) value, the retention time of each analyte compound in the sample was compared and calculated with the retention time of a series of n-alkane compounds from C8—C40 injected by GC-MS using the same procedure as the GC-MS method for sample analysis.

2.2.8. GC-MS Injection

The volatiles were analyzed by GC-MS (GC 2010 GCMS-QP2010 plus, Shimadzu, Japan) using a previously reported technique (24).The other detailed settings and specifications are listed in Table 2.

Table 2. GC-MS settings and specifications.

Specification	Information
Injection port	Splitless; temp. 175 ℃
Column type	Rtx-5MS 30 m, 0.25 mm ID, 0.25 µm
Initial oven temperature	33 ℃, 5 minutes hold time
Oven temperature increase	10 ℃/min
Final oven temperature	200 ℃, 5 minutes hold time
Transfer line temperature	200 ℃
MS quadrupole	150 ℃
Iron source temperature	230 ℃
Total analysis time	26 minutes

2.2.9. Statistical and Multivariate Data Analysis

The data of physicochemical characteristics of the patties were analyzed using IBM SPSS Statistics 24 and Minitab 20 with two-way ANOVA. The Bonferroni test and pairwise comparisons were conducted when the data obtained differed significantly ($\alpha < 0.05$). The analysis was carried out with 3 repetitions without any replication. The experimental methodology chosen for this study was a Completely Randomized Factorial Design with the treatment factors of meat content (A) and seasoning (B).

The GC-MS raw data, which consisted of compounds' names and peak area integration, were converted into data matrices that included sample information as well as the relative intensities of each chemical. The GC-MS metabolite mass spectra were manually glossed using the Chemstation E. 02.02.1431 output and the NIST14 Mass Spectral Library. The LRI of each annotated metabolite was determined by comparing its retention time on the RTX-5MS column to the retention time of the standard alkane solution. SIMCA software (v.16.0, Sartorius-Umetric, Umea, Sweden) was used to analyze the volatile compounds obtained by PCA and OPLS-DA. Pareto scaling was used to lower the relative relevance of large values while retaining some of the data structure. Instead of unit variance, this provides the variable a variance equal to its standard deviation. Response permutation tests and cross validation were used to validate the PCA and OPLS-DA models. They were indicated by Q^2 which had to be at least 0.4 in value. When the Q^2 values in the permutation test were greater than Q^2 obtained by random models using the identical datasets, the data model was declared trustworthy. To find the significant differentiating compounds in each group, the VIP and coefficient correlation values were used. The valid discriminating compounds are those with VIP ≥ 1 and positive correlation coefficient value.

3. Results and Discussion

3.1. Proximate Analysis

The results of the proximate analysis (Table 3) show that increasing the beef content in patties raises the ash, total fat, and protein levels. When comparing unseasoned and seasoned patties, unseasoned samples had lower ash and moisture contents, but higher total fat and protein levels. The ash content observed in this study was consistent with previous findings. The addition of salt and garlic to beef patties and dried smoked beef has been reported to increase the ash content compared to control samples without these seasonings (25,26). As the ash content reflects the mineral content of the sample, this increase was significant. A comparative mineral analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) revealed that roasted beef contains higher levels of minerals than roasted pork—particularly aluminum (Al), barium (Ba), calcium (Ca), potassium (K), iron (Fe), copper (Cu), sodium (Na), and zinc (Zn) (27).

The moisture content was higher in samples containing more pork than in those with higher beef content (Table 3). This may be attributed to pork's lower fat content, as fat tends to displace water. Additionally, the presence of spices appeared to increase the moisture content of the samples. This aligns with previous studies reporting increased moisture in roasted beef patties after the addition of spices such as rosemary, turmeric, and bay leaf. It is believed that bioactive compounds in these spices—particularly phenolic compounds and essential oils—help retain water, although the exact mechanism remains unclear (28). The total fat content was higher in samples with more beef than those with more pork (Table 3),

which is consistent with the fact that beef generally contains more total fat than pork (29). Furthermore, beef has a higher proportion of saturated fatty acids, which have higher melting points compared to unsaturated fatty acids found in pork. As a result, pork-containing samples tend to have a lower total fat content after cooking because of fat melting. The addition of spices also contributed to a reduction in the total fat content, supporting this observation.

3.2. Color Analysis

Table 3 shows that as pork content in the patty increases, so do the L*, C*, and h* values. Meat color is influenced by several factors, including heme pigment concentration (e.g., myoglobin), the state of the heme pigment, meat characteristics (such as fiber type) (30), non-meat ingredients, and added food additives (31).Myoglobin content is also associated with the predominant muscle fiber type (32). Meat with a higher proportion of Type I fibers—such as beef—has higher myoglobin content. Higher myoglobin levels are generally associated with lower L* values, indicating a darker color. Since beef contains more Type I fibers than pork, it also has a higher myoglobin content, explaining why patties with less beef (and more pork) had higher L* values. Meat with a higher proportion of Type I fibers—such as beef—has higher myoglobin content. Higher myoglobin levels are generally associated with lower L* values, indicating a darker color. Previous studies have reported that the C* and h* values of meat are related to the redox state of myoglobin. Greater h* values are linked to increased oxymyoglobin formation, while higher C* values indicate more metmyoglobin accumulation (33). In our study, higher pork concentrations were associated with decreases in both C* and h* values, suggesting more intense myoglobin oxidation in patties with a higher percentage of pork. This may be due to pork's higher content of unsaturated fatty acids compared to beef, as unsaturated fats are more susceptible to lipid oxidation (34).

Table 3 also shows that the seasoned patties generally had lower L* and high h* values, whereas C*was not significantly different from that of the unseasoned patties. The addition of spices such as garlic, turmeric, and rosemary were found to reduce the lightness of cooked chicken wing and pork belly (31). Salt addition reduced the L* value (35). This is because salt can oxidize myoglobin and change it from a bright red to a brownish color, as it is a well-known oxidizer of myoglobin. These results support the results obtained from the research which state that the lightness of the patty sample decreases with the addition of salt. Garlic has been demonstrated to have significant antioxidant activity, controlled rancidity and lipid oxidation when added to ground beef (36) which is mostly attributed to its rich concentration of organosulfur compounds and their precursors, thus preventing meat discoloration.

3.3. Water Holding Capacity and Cooking Loss

Table 3 further shows that water-holding capacity increased and cooking loss decreased as the beef content in the patties rose. The ability of meat to retain moisture depends on several factors, one of which is the type of muscle fiber. There are three main types of muscle fibers: type I, type IIa, and type IIb. Beef contains a higher proportion of type I fibers compared to pork. Meat rich in type I fibers, such as beef, generally retains water more effectively (32). When comparing unseasoned patties to seasoned ones, the unseasoned patties exhibited lower water-holding capacity but higher cooking loss. This finding is consistent with previous studies showing that the addition of spices to beef patties significantly reduces cooking loss

(28).It is suggested that antioxidant compounds in spices help maintain the structural integrity of muscle membranes, thereby preventing moisture loss during cooking. Additionally, higher salt content has been shown to improve water-holding capacity by promoting myofibrillar swelling (37). In the presence of salt, part of the otherwise insoluble myosin dissolves into the liquid phase, enhancing meat swelling and water retention due to the dissociation of ions (H^+ and OH^-). Salt-solubilized myofibrillar proteins create a sticky exudate that forms a heat-coagulated protein matrix during cooking, effectively trapping free water and improving overall moisture retention (38).

3.4. Texture Analysis

The results of this study showed that the hardness, cohesiveness, and chewiness of the sample increased as the beef content in the patties increased (Table 3). In contrast, the springiness of the samples did not appear to be significantly different at different meat ratios. In contrast, the unseasoned sample had higher hardness, springiness, cohesiveness, and chewiness than the seasoned sample. The differences in hardness, cohesiveness, and chewiness between beef and pork can be attributed to variations in muscle structure and fiber type. As mentioned above, beef contains a higher proportion of Type I muscle fibers, which are denser and more fatigue-resistant. In contrast, pork has more Type II fibers, which are generally less dense and more tender (32). Because Type I fibers are more compact and rich in collagen, beef tends to be firmer and tougher, resulting in greater hardness and chewiness. Meat texture correlates with its water holding capacity (32). Beef patties have a higher water holding capacity, therefore, they retains water better due to muscle fiber composition, contributing to a more cohesive texture. In contrast, pork loses more moisture during cooking, which can reduce cohesiveness and make the soften texture.

Table 3. Physicochemical characteristics of samples with different ratios beef and pork.

Parameter	Seasoning	100_0	75_25	50_50	25_75	0_100
Proximate Analysis						
Ash	Unseasoned	1.85 ± 0.04^{Aa}	1.32 ± 0.01^{Ab}	1.39 ± 0.04^{Ab}	1.11 ± 0.03^{Abc}	1.14 ± 0.03^{Ac}
	Seasoned	2.61 ± 0.07^{Ba}	2.59 ± 0.06^{Bb}	2.49 ± 0.08^{Bb}	2.64 ± 0.03^{Bbc}	2.36 ± 0.06^{Bc}
Moisture	Unseasoned	57.98 ± 0.21^{Ad}	66.21 ± 0.11^{Ac}	67.48 ± 0.45^{Ab}	69.23 ± 0.53^{Aa}	67.92 ± 0.71^{Aab}
	Seasoned	65.14 ± 0.05^{Bd}	67.50 ± 0.25^{Bc}	68.40 ± 0.26^{Bb}	69.23 ± 0.02^{Ba}	69.35 ± 0.19^{Bab}
Total fat	Unseasoned	7.74 ± 0.02^{Aa}	5.81 ± 0.01^{Ab}	4.47 ± 0.01^{Ae}	4.25 ± 0.01^{Ad}	4.48 ± 0.05^{Ac}
	Seasoned	5.86 ± 0.06^{Ba}	4.87 ± 0.06^{Bb}	3.22 ± 0.04^{Be}	4.44 ± 0.02^{Bd}	4.50 ± 0.04^{Bc}
Protein	Unseasoned	31.38 ± 0.25^{Aa}	25.62 ± 0.11^{Abc}	25.64 ± 0.50^{Ab}	24.40 ± 0.54^{Ac}	25.46 ± 0.69^{Abc}
	Seasoned	25.38 ± 0.18^{Ba}	22.39 ± 0.23^{Bbc}	23.75 ± 0.06^{Bb}	22.74 ± 0.02^{Bc}	22.78 ± 0.16^{Bbc}
Color						
L* (%)	Unseasoned	55.46 ± 0.16^{Ad}	57.59 ± 1.58^{Ac}	58.89 ± 1.41^{Ac}	64.36 ± 1.14^{Ab}	66.32 ± 0.72^{Aa}
	Seasoned	53.96 ± 0.29^{Bd}	57.44 ± 1.72^{Bc}	57.82 ± 1.12^{Bc}	59.50 ± 2.04^{Bb}	64.25 ± 1.53^{Ba}
C* (%)	Unseasoned	12.70 ± 0.44^{Ac}	13.22 ± 0.24^{Ab}	14.65 ± 0.59^{Aa}	14.88 ± 0.39^{Aa}	15.05 ± 1.05^{Aa}
	Seasoned	11.43 ± 0.50^{Ac}	13.88 ± 0.29^{Ab}	14.75 ± 0.53^{Aa}	14.84 ± 0.90^{Aa}	14.45 ± 0.27^{Aa}
h (°)	Unseasoned	58.90 ± 2.48^{Ad}	59.05 ± 2.47^{Ac}	63.66 ± 2.27^{Ab}	64.92 ± 1.97^{Aab}	70.83 ± 1.06^{Aa}
	Seasoned	59.71 ± 1.16^{Bd}	67.39 ± 2.60^{Bc}	69.51 ± 1.07^{Bb}	70.61 ± 0.81^{Bab}	70.48 ± 2.46^{Ba}
Texture						
Hardness (g)	Unseasoned	8867.65 ± 610.05^{Aa}	7983.63 ± 126.92^{Ab}	6587.98 ± 644.48^{Ac}	6145.08 ± 408.69^{Ad}	5309.35 ± 132.27^{Ad}
	Seasoned	7161.23 ± 246.13^{Ba}	6528.38 ± 291.92^{Bb}	5739.85 ± 164.76^{Bc}	5037.13 ± 312.66^{Bd}	4784.75 ± 236.82^{Bd}

Parameter	Seasoning	100_0	75_25	50_50	25_75	0_100
Proximate Analysis						
Springiness (%)	Unseasoned	0.28±0.02^{Aa}	0.29±0.02^{Aa}	0.30±0.04^{Aa}	0.29±0.02^{Aa}	0.27±0.01^{Aa}
	Seasoned	0.22±0.02^{Ba}	0.23±0.02^{Ba}	0.22±0.01^{Ba}	0.22±0.01^{Ba}	0.23±0.03^{Ba}
Cohesiveness (%)	Unseasoned	0.45±0.02^{Aa}	0.44±0.02^{Aa}	0.42±0.02^{Ab}	0.42±0.03^{Aab}	0.38±0.02^{Ac}
	Seasoned	0.44±0.02^{Aa}	0.44±0.02^{Aa}	0.39±0.01^{Ab}	0.41±0.03^{Aab}	0.36±0.03^{Ac}
Chewiness (gmm)	Unseasoned	1099.89±145.69^{Aa}	1019.38± 85.70^{Aa}	825.02±138.42^{Ab}	767.17±135.90^{Ab}	548.16±41.22^{Ac}
	Seasoned	705.64± 65.79^{Ba}	646.03±50.01^{Ba}	495.69±28.87^{Bb}	455.32±65.52^{Bb}	394.06±22.10^{Bc}
Water Holding Capacity						
	Unseasoned	49.23±1.42^{Aa}	20.02±3.69^{Ab}	19.39±1.93^{Ab}	17.34±1.53^{Ab}	30.13±3.20^{Ab}
	Seasoned	54.96±3.59^{Ba}	48.76±1.82^{Bb}	47.88±2.20^{Bb}	48.38±4.69^{Bb}	37.12±1.18^{Bb}
Cooking Loss						
	Unseasoned	35.51±0.61^{Aa}	35.48±0.90^{Aa}	33.18±2.59^{Ab}	28.91±0.87^{Ac}	24.66±1.73^{Ad}
	Seasoned	32.43±0.24^{Ba}	31.23±0.32^{Ba}	28.68±1.27^{Bb}	25.37±0.87^{Bc}	23.49±0.31^{Bd}

* For each parameter, the values in the column followed by the same majuscule are not statistically different at α = 0.05. For each parameter, the values in the row followed by the same minuscule are not statistically different at α = 0.05. This finding has a significance of α = 0.05.

3.5. Volatilomics

A total of 167 compounds were detected in the samples (Table 4) and were classified into various groups: organic acids, alcohols, aldehydes, alkanes, alkenes, alkyl thiols, aromatic hydrocarbons, esters, heterocyclics, ketones, nitrogen- and sulfur-containing compounds, and terpenoids. Heating meat triggers multiple chemical reactions—such as lipid oxidation, the Maillard reaction, Strecker degradation, and the breakdown of thiamine and carbohydrates. Interactions between these reactions and their byproducts result in changes to the chemical composition of the meat. These processes generate volatile compounds—including alcohols, hydrocarbons, ketones, aldehydes, esters, carboxylic acids, and various halogenated and sulfur-containing molecules, which contribute to the characteristic beef flavor (8,17).

Table 4. Volatile compounds identified in beef, pork, and their mixtures with various ratios using SPME/GC-MS.

Compound	CAS	LRI	Identification Method*
Acids			
Butyric acid, 2-phenyl-, undec-2-en-1-yl ester	-	972.6	M
Butanoic acid, anhydride	106-31-0	1046.7	M
Butanoic acid, 3-methylbutyl ester	106-27-4	1061.1	L
cis-Chrysanthenyl formate	241123-18-2	1143.9	L
Vanillic Acid, 2TMS derivative	2078-15-1	1223.9	L
Isovanillic acid, 2TMS derivative	68595-68-6	1224.0	L
Hexanethioic acid, S-propyl ester	2432-78-2	1249.6	L
Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	77-68-9	1387.9	L
Carbamodithioic acid, diethyl-, methyl ester	0686-07-07	1406.6	M
Octanoic acid, 3-methylbutyl ester	2035-99-6	1453.9	L
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	-	1610.1	M
Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	55191-44-1	1721.2	M

Compound	CAS	LRI	Identification Method*
Alcohols			
Ethanol	64-17-5	448.0	M
1-Pentanol	71-41-0	768.0	M
1-Heptanol	111-70-6	980.2	L
1-Octen-3-ol	3391-86-4	984.9	L
Eucalyptol	470-82-6	1037.4	L
2-Decen-1-ol, (E)-	18409-18-2	1079.3	M
p-Mentha-1,5-dien-8-ol	1686-20-0	1172.5	L
Terpinen-4-ol	562-74-3	1187.8	L
5,7-Octadien-2-ol, 2,6-dimethyl-	5986-38-9	1203.9	L
1,3-Propanediol, ethyl tetradecyl ether		1204.5	M
E-2-Tetradecen-1-ol	-	1720.8	M
1-Dodecanol, 3,7,11-trimethyl-	6750-34-1	1787.2	M
Aldehydes			
Pentanal	110-62-3	702.0	M
Hexanal	66-25-1	800.0	M
Heptanal	111-71-7	901.7	L
2-Heptenal, (E)-	18829-55-5	963.6	L
Octanal	124-13-0	1008.1	L
Benzeneacetaldehyde	122-78-1	1055.9	L
2-Octenal, (E)-	2548-87-0	1066.2	L
Nonanal	124-19-6	1109.4	L
Decanal	112-31-2	1212.2	L
2-Decenal, (E)-	3913-81-3	1273.0	L
2,4-Dodecadienal, (E,E)-	21662-16-8	1307.7	M
Undecanal	112-44-7	1314.3	L
Dodecanal	112-54-9	1419.6	L
Tridecanal	10486-19-8	1519.4	L
Tetradecanal	124-25-4	1824.4	L
Pentadecanal-	316249.0	1824.8	L
Heptadecanal	629-90-3	1825.3	L
Alkanes			
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	18172-67-3	976.3	L
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	18172-67-3	978.8	L
Octane, 3,3-dimethyl-	4110-44-5	1023.4	M
Undecane, 5,7-dimethyl-	17312-83-3	1059.7	M
Undecane, 4,7-dimethyl-	17301-32-5	1059.8	M
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α,5α)-	17699-16-0	1128.7	L
Hexadecane	544-76-3	1200.7	L
Dodecane	112-40-3	1200.8	M
Undecane, 2-methyl-	7045-71-8	1201.1	L
Decane, 2,6,7-trimethyl-	62108-25-2	1214.7	M
Eicosane, 2,4-dimethyl-	75163-98-3	1264.6	M
Undecane, 2,4-dimethyl-	17312-80-0	1264.9	L
Octane, 6-ethyl-2-methyl-	62016-19-7	1294.1	M
Tridecane	629-50-5	1299.7	L
Methane, (methylsulfinyl)(methylthio)-	33577-16-1	1308.1	L
Dodecane, 4-methyl-	6117-97-1	1326.3	L
Decane, 2,3,7-trimethyl-	62238-13-5	1326.7	M
Dodecane, 4,6-dimethyl-	61141-72-8	1327	L

Compound	CAS	LRI	Identification Method*
Nonane, 5-butyl-	17312-63-9	1335.3	M
1-Undecene, 4-methyl-	74630-39-0	1335.8	M
Decane, 3,7-dimethyl-	17312-54-8	1336.1	L
Tridecane, 4-methyl-	9069307.0	1359.6	L
Tetradecane	629-59-4	1402.9	L
Undecane, 4,8-dimethyl-	17301-33-6	1424.8	M
Heptadecane	629-78-7	1425.6	L
Tetradecane, 1-chloro-	2425-54-9	1454.6	M
Octadecane, 1-chloro-	3386-33-2	1454.6	M
Nonadecane	629-92-5	1454.6	L
Heneicosane, 11-(1-ethylpropyl)-	19497687.0	1455.1	M
Heneicosane	629-94-7	1460.3	M
1-Nonene, 4,6,8-trimethyl-	54410-98-9	1464.2	M
Dodecane, 2,6,10-trimethyl-	3891-98-3	1464.5	L
2,6,10-Trimethyltridecane	3891-99-4	1464.8	L
Octadecane, 5-methyl-	25117-35-5	1496.9	M
Hexane, 3,3-dimethyl-	563-16-6	1497.7	M
Dodecane, 2,6,11-trimethyl-	31295-56-4	1497.9	M
Heptadecane, 2,6,10,15-tetramethyl-	54833-48-6	1499.0	M
Pentadecane	629-62-9	1501.0	L
Undecane, 2,3-dimethyl-	17312-77-5	1501.4	M
Eicosane	112-95-8	1708.8	L
Alkenes			
1,3-Hexadiene, 3-ethyl-2-methyl-	61142-36-7	1039.4	L
Azulene	275-51-4	1198.0	M
3-Vinyl-1,2-dithiacyclohex-4-ene	62488-52-2	1201.2	L
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	118-65-0	1441.2	L
1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene	-	1508.1	M
Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1α,7α,8aβ)]-	3691-11-0	1517.5	L
(3R,4aS,8aS)-8a-Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	212394-95-1	1654.6	L
(1S,7S,8aR)-1,8a-Dimethyl-7-(prop-1-en-2-yl)-1,2,3,7,8,8a-hexahydronaphthalene	190327-38-9	1655.4	L
2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	14237-73-1	1844.7	L
Alkylthiols			
Methanethiol	74-93-1	464.0	M
Aromatic Hydrocarbons			
Ethylbenzene	100-41-4	836.7	L
o-Xylene	95-47-6	849.7	L
Benzene, 1,3-dimethyl-	108-38-3	849.8	L
Thiophene, 3,4-dimethyl-	632-15-5	901.8	L
Pyrazine, 2,5-dimethyl-	123-32-0	911.3	L
Benzene, 1,2,4-trimethyl-	95-63-6	996.2	L
Mesitylene	108-67-8	997.2	L
Benzene, 1,2,3-trimethyl-	526-73-8	997.6	L
Benzene, 1,3-dichloro-	541-73-1	1019.1	L
Benzene, (2,2-dimethylbutyl)-	28080-86-6	1060.9	M

Compound	CAS	LRI	Identification Method*
Benzene, 1,2,4,5-tetramethyl-	95-93-2	1126.8	L
Naphthalene	91-20-3	1198.6	L
Diphenyl ether	101-84-8	1424.0	L
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]-	473-13-2	1509.3	L
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]-	17066-67-0	1509.6	L
Phenol, 3,5-bis(1,1-dimethylethyl)-	1138-52-9	1526.4	L
Butylated Hydroxytoluene	128-37-0	1527.3	L
Benzene, (1-butylhexyl)-	963455.0	1547.4	L
Benzene, (1-propylheptyl)-	963486.0	1556.3	M
Benzene, (1-pentylhexyl)-	4537-14-8	1640.9	M
Benzene, (1-butylheptyl)-	4537-15-9	1644.9	L
Benzene, (1-ethylbutyl)-	4468-42-2	1654.9	L
Benzene, (1-propyloctyl)-	4536-86-1	1655.1	L
Esters			
4-Methylpentyl 4-methylpentanoate	35852-42-7	1042.3	L
Allyl heptanoate	142-19-8	1185.2	L
Propyl octanoate	624-13-5	1295.5	M
Methyl 2-hydroxystearate, TMS derivative	56196-58-8	1366.7	M
4-tert-Butylcyclohexyl acetate	-655852.0	1382.5	M
Heterocyclics			
Furan, 2-pentyl-	3777-69-3	995.1	L
2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	56599-50-9	1441.3	M
Ketones			
Acetoin	513-86-0	-	M
2-Heptanone	110-43-0	887.8	L
Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	-257124.0	1337.2	M
2,5-di-tert-Butyl-1,4-benzoquinone	2460-77-7	1483.8	M
Nitrogen compounds			
Trisulfide, di-2-propenyl	2050-87-5	1315.8	L
Sulfur Compounds			
Carbon disulfide	75-15-0	-	M
Thiirane, methyl-	1072-43-1	-	M
Disulfide, dimethyl	624-92-0	-	M
Sulfide, Diallyl	592-88-1	827.8	L
Sulfide, allyl propyl;	27817-67-0	853.3	L
(Z)-Allyl(prop-1-en-1-yl)sulfane	104324-69-8	885.4	L
Disulfide, methyl 2-propenyl	2179-58-0	916.6	L
Diallyl disulphide	2179-57-9	1090.1	L
1-Allyl-2-isopropyldisulfane	67421-85-6	1099.1	L
(E)-1-Allyl-2-(prop-1-en-1-yl)disulfane	122156-02-9	1106.7	L
2-Ethyl[1,3]dithiane	6007-23-4	1122.2	M
(E)-1-(Prop-1-en-1-yl)-2-propyldisulfane	23838-21-3	1122.3	L
Trisulfide, methyl 2-propenyl	34135-85-8	1148.5	L
α-Terpineol	98-55-5	1203.4	L
2-Vinyl-4H-1,3-dithiine	80028-57-5	1228.6	L
1-Allyl-3-propyltrisulfane	33922-73-5	1327.2	L

Compound	CAS	LRI	Identification Method*
(E)-1-Allyl-3-(prop-1-en-1-yl)trisulfane	382161-78-6	1343.6	L
Terpenoids			
α-Pinene	80-56-8	932.7	L
trans-β-Ocimene	3779-61-1	933.7	L
β-Myrcene	123-35-3	994.1	L
α-Phellandrene	99-83-2	1007.1	L
3-Carene	13466-78-9	1012.6	L
2-Carene	554-61-0	1019.1	L
p-Cymene	99-87-6	1028.8	L
o-Cymene	527-84-4	1030.0	L
D-Limonene	5989-27-5	1032.7	L
β-Ocimene	13877-91-3	1052.2	L
γ-Terpinene	99-85-4	1063.8	L
(+)-4-Carene	29050-33-7	1094.0	L
Linalool	78-70-6	1104.2	L
cis-Dihydrocarvone	3792-53-8	1217.5	L
Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, [1S-(1α,3β,5α)]-	471-16-9	1217.7	L
Copaene	3856-25-5	1391.5	L
Ylangene	14912-44-8	1392.2	L
β-Bisabolene	495-61-4	1392.8	L
(3R,3aR,7R,8aS)-3,8,8-Trimethyl-6-methyleneoctahydro-1H-3a,7-methanoazulene	79120-98-2	1425.9	L
Caryophyllene	87-44-5	1440.6	L
α-Guaiene	654486.0	1455.1	L
Humulene	6753-98-6	1474.4	L
Caryophyllene oxide	1139-30-6	1613.6	L
(+)-3-Carene, 10-(acetylmethyl)-	-	1656.0	L

**Identifications method for volatile compounds:*
L: Compound identified from similar mass spectra on NIST 14 Library Chemistry Webbook and have similar LRI with similar compounds on data available in the GC-MS databank.
M: Compound identified from similar mass spectra on NIST 14 Library Chemistry Webbook

The volatilome data was subjected to multivariate data analysis to identify potential markers for different type of patties. Figure 1 presents the PCA scatter score plot of the volatile compound data from all patty samples. The PCA model explained 78.3% of the total variation (R^2X[cum] = 0.783) with a Q^2[cum] of 0.678, indicating that the model was reliable (40). The plot clearly shows that seasoned and unseasoned patties formed two distinct clusters, suggesting that seasoning has a significant impact on the volatile profiles. This finding aligns with previous studies reporting that spices such as nutmeg, garlic, onion, and ginger can substantially alter the volatile composition of cooked patties. Given the clear differences between seasoned and unseasoned samples, further analysis focused on the all-beef, all-pork, and mixed patties within each category. Separate PCA and OPLS-DA models were developed for the seasoned and unseasoned groups, and the results are shown in Figure 2.

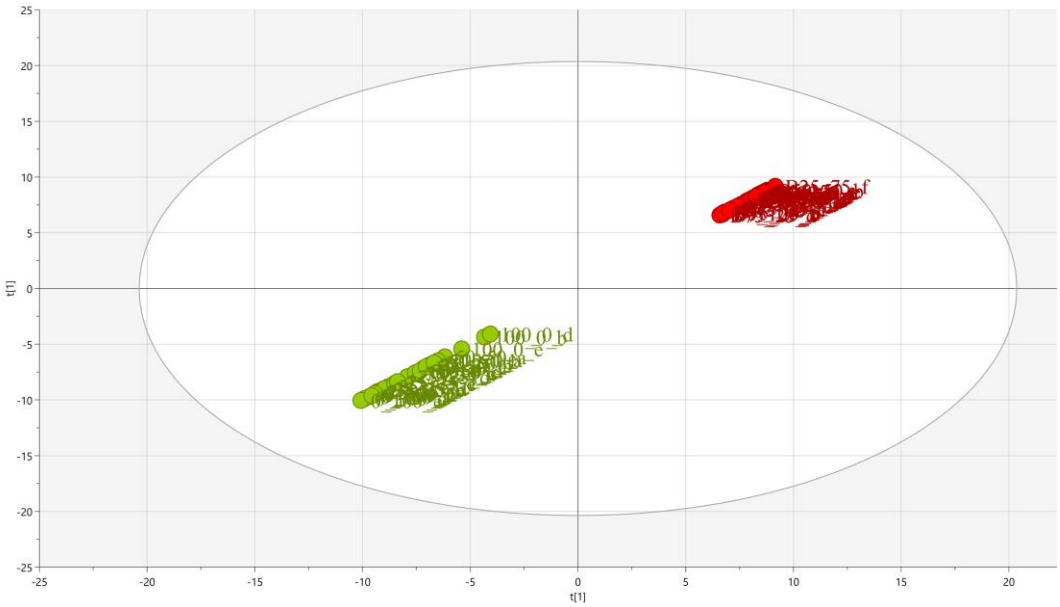

Figure 1. PCA scatter plot of volatiles data of all samples; unseasoned patty (green) and seasoned patty (red).

Figure 2a shows the PCA scatter score plot of the unseasoned samples. The PCA model explained 57.0% of the total variation ($R^2X(cum) = 0.570$) with a $Q^2(cum)$ of 0.319. Although the Q^2 value was slightly below the commonly accepted threshold of 0.4, it was still considered reasonably close. The sample grouping was clearly defined as follows: pure beef samples (green) clustered in the third quadrant, pure pork samples (red) grouped between the first and second quadrants, and mixed samples (blue) positioned between the pure beef and pure pork clusters. Figure 2b displays the OPLS-DA scatter score plot for the seasoned samples, which included pure beef, pure pork, and their mixtures in various ratios. The OPLS-DA model explained 71.4% of the total variation ($R^2X(cum) = 0.714$) with a $Q^2(cum)$ of 0.878, indicating high model reliability (39). The plot shows three distinct groupings: pure beef samples (green) in the second quadrant, pure pork samples (red) in the third quadrant, and mixed meat samples (blue) in the fourth quadrant. Notably, the mixed samples—comprising 75% beef/25% pork, 50% beef/50% pork, and 25% beef/75% pork—were all clearly separated from the pure meat groups. These results demonstrate that even the addition of as little as 25% pork to beef patties is sufficient to distinguish them from all-beef patties based on their volatile compound profiles.

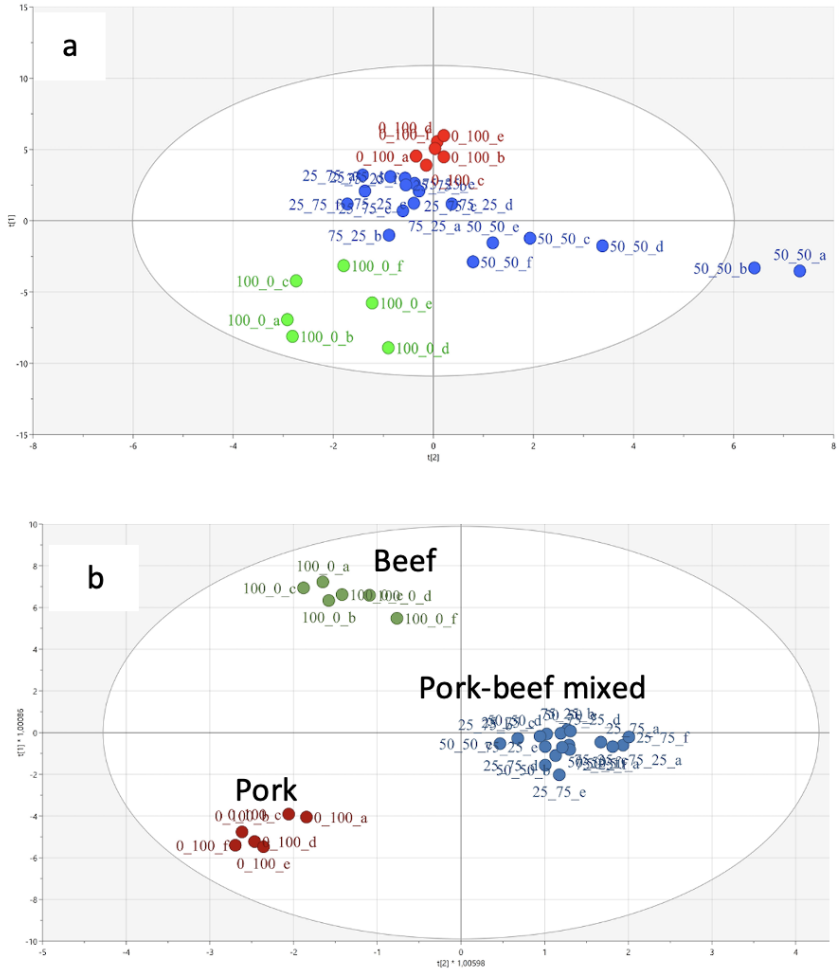

Figure 2. (a) PCA score plot of unseasoned patty volatile data. Red, green, and blue represent all pork patties, all beef patties, and mixed patties, respectively. (b) OPLS-DA score plot of unseasoned patty volatile data divided into 3 classes based on the beef and pork ratio: all-pork patty (red), all-beef patty (green), and mixed patty (blue).

Figure 3a shows the PCA scatter score plot of samples consisting of pure beef, pure pork, and their mixtures in varying ratios, all combined with seasonings. The sample grouping was fairly distinct, although the samples containing 25% beef and 75% pork appeared closer to the 100% pork group. The PCA model explained 65.4% of the total variation ($R^2X(cum)$ = 0.654), with a $Q^2(cum)$ value of 0.337. Although the Q^2 value is below the commonly accepted reliability threshold of 0.4, it is still considered sufficiently close enough to allow meaningful grouping of the samples (39). Figure 3b shows the OPLS-DA scatter score plot for the same set of seasoned samples. The model explained 82.6% of the total variation ($R^2X(cum)$ = 0.826), with a $Q^2(cum)$ value of 0.680, indicating good model reliability (39). The OPLS-DA plot displayed clear separations among the predefined groups: pure beef samples (green) clustered in the second quadrant, pure pork samples (red) in the first quadrant, and mixed samples (blue) positioned between the third and fourth quadrants. All mixed-ratio samples were distinctly separated from the pure meat groups. These results indicate that, even in the presence of seasonings, patties made from pure beef, pure pork, and their mixtures can still be reliably distinguished based on their volatile compound profiles.

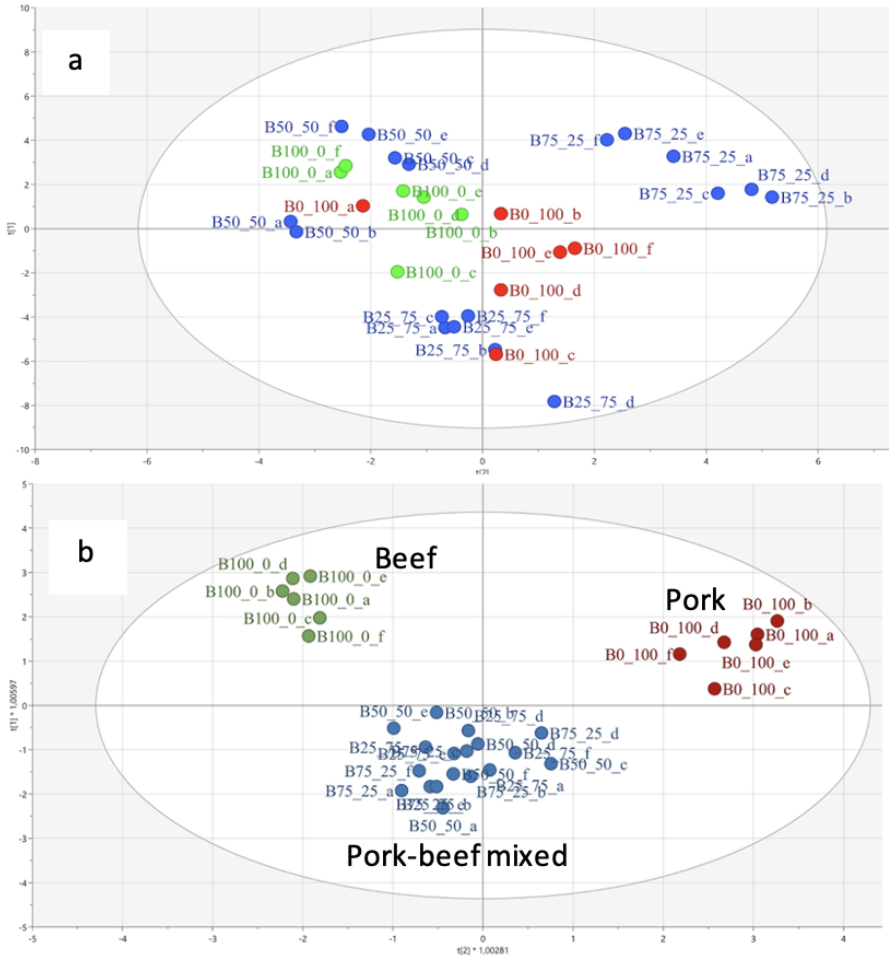

Figure 3. (a) PCA score plot of seasoned patty volatile data. Red, green, and blue represent all pork patties, all beef patties, and mixed patties, respectively. (b) OPLS-DA score plot of seasoned patty volatile data divided into 3 classes based on the beef and pork ratio: all pork patty (red), all-beef patty (green), and mixed patty (blue).

As shown in Table 5, Dimethyl disulfide, Hexanal, and Naphthalene were identified as potential volatile markers for pure beef patties, pure pork patties, and mixed patties, respectively. In the unseasoned samples, 3,7,11-Trimethyl-1-dodecanol, Hexadecane, and Nonanal were identified as marker compounds for pure beef patties. For pure pork patties, Hexanal, (E)-2-Octenal, (E)-2-Heptenal, and 2-Pentyl-furan served as potential markers. Mixed patties were characterized by the presence of Naphthalene, Octanal, and Heptanal. Notably, each category—beef, pork, and mixed—had a unique set of marker compounds. These findings confirm that pure beef and pure pork patties possess distinct volatile profiles. Moreover, blending different types of meat alters the overall volatile composition, resulting in a unique profile for mixed patties.

Table 5. Compounds with positive coefficient values and VIP value ≥1 as potential volatile marker selected from each OPLS-DA class of pure beef patty, pure pork patty, and their mixture.

Compound	Class	Coefficient	VIP	Chemical Group
Seasoned Samples				
Dimethyl disulfide	Beef	0.1421	1.0041	Sulphuric Compounds
Naphthalene	Beef-Pork Mixed	0.2611	3.0650	Aromatic Hydrocarbons
Hexanal	Pork	0.2130	1.5431	Aldehydes
Unseasoned Samples				
3,7,11-Trimethyl-1-dodecanol	Beef	0.1357	1.6279	Alcohols
Hexadecane		0.0823	1.0484	Alkanes
Nonanal		0.0709	1.0487	Aldehydes
Naphthalene	Beef-Pork	0.2372	1.4810	Aromatic Hydrocarbons
Octanal	Mixed	0.1732	1.3684	Aldehydes
Heptanal		0.1448	1.1344	Aldehydes
Hexanal	Pork	0.1059	4.2306	Aldehydes
(E)-2-Octenal		0.2224	1.6142	Aldehydes
(E)-2-Heptenal		0.2214	1.5281	Aldehydes
2-Pentyl- furan		0.1559	1.2280	Heterocyclics

Benzaldehyde, 1-Pentanol, Hexanal, Octanal, Nonanal, Decanal, Dodecanal, Tridecanal, Pentadecanal, Hexadecanal, and Acetoin have been identified as key volatile compounds in beef roasted for different times (40). Dimethyl disulfide and Hexadecane are also found in beef meatballs (41), while Naphthalene has been detected in roasted beef (41),and Naphthalene is present in roasted beef (42). Additionally, 3,7,11-Trimethyl-1-dodecanol has been reported in beef subjected to various curing treatments (43). Hexadecane is a major hydrocarbon in both beef grease (44) and pork protein (45). Heptanal, Octanal, (E)-2-Octenal, (E)-2-Heptenal, and 2-Pentyl-furan are recognized as major volatile compounds in pork (46,47). In samples containing seasonings, fewer potential volatile marker compounds were identified compared to unseasoned samples. As discussed earlier, seasonings—particularly garlic—significantly alter the volatile profile owing to their reactive bioactive compounds. These compounds may interact with those in the meat, either directly or with the assistance of thermal processing, leading to changes in detectable volatiles (48).Some marker compounds present in unseasoned samples may be absent in seasoned samples because of these interactions. Interestingly, 3,7,11-Trimethyl-1-dodecanol has been detected in both beef (49) and garlic (50) supporting its identification as a potential marker in seasoned beef samples. Hexanal was found at higher concentrations in seasoned pork patties, likely due to its role in promoting oxidative processes in the meat matrix (51).

4. Conclusions

The ratio of meat and the addition of seasonings influence both the physicochemical and volatilome characteristics of patties made from beef, pork, and their mixtures. Through this analysis, the potential volatile marker compounds were identified. Dimethyl disulfide was found to be a potential marker for pure beef patties, hexanal for pure pork patties, and naphthalene for mixed patties. In unseasoned samples, 3,7,11-trimethyl-1-dodecanol, hexadecane, and nonanal served as markers for pure beef patties; hexanal, (E)-2-octenal, (E)-2-heptenal, and 2-pentyl-furan for pure pork patties; and naphthalene, octanal, and heptanal

for mixed patties. These findings demonstrated that physicochemical and volatilome analyses can support the differentiation of halal patties (made from beef) from non-halal patties (containing pork or beef-pork mixtures). However, further chemical validation is required to confirm the presence of these volatile markers. This should include quantification by using authentic reference standards.

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Author Contributions

N.D.Y., D., and A.R. conceived and designed the experiments; M.K.R. performed the experiments and wrote the first paper; D.I., F.S.B., and Y.R. analyzed the data; N.K.A.B. contributed reagents and materials. All authors read and approved the manuscript.

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Institutional Review Board Statement

Not applicable.

Data Availability Statement

Not applicable.

Conflicts of Interest

Author discloses no conflict of interest.

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