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Research article

Volatile compounds, texture, and color characterization of meatballs made from beef, rat, wild boar, and their mixtures

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ABSTRACT

The purpose of this research was to characterize the volatile compounds, texture, and color profile of meatballs made from beef, rat, wild boar, and their combinations. Volatile compounds were analyzed using SPME/GC-MS and multivariate data analysis (PCA, PLS-DA). Additionally, several textural features such as hardness, gumminess, chewiness, cohesiveness, and colour (L, a*, b*, C, and h) were also analyzed. The findings revealed that texture and color characteristics can only be used to differentiate meatballs based on their raw meat materials when meat adulterants are used in high concentrations (≥50%). PLS-DA analysis of volatile data revealed distinct groupings among various types of meatballs, including meatballs adulterated with rat or wild boar meat at the lowest percentage used in this study (20%). By using VIP and correlation coefficient, 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. Nonanal was consistently found as a significant marker in the meatballs made from a mixture of beef-rat and beef-wild boar at different ratios. This study demonstrated that the volatile profile of meat is more reliable than physicochemical profiles for developing an analytical tool for quickly identifying undesired meat in meat-derived products.

1. Introduction

Meatballs are one of Indonesia's most popular street food. The high price of beef triggers the adulteration of beef with other meats with cheaper price, such as pork, horses, wild boars, and even rat meat. Meat adulteration cases frequently occur in Indonesia. The case is very sensitive when the adulterant is from non-halal animals, since Indonesia is one of the largest Muslim populations in the world. Wild boar is used for food and sport hunting all over the world [1, 2]. The recent increase in natural populations and the potential of farming wild boars have stimulated interest in this species as a meat source [1]. Wild boar is frequently used as a meat adulterant because the price is significantly cheaper than beef. Even worse, in Indonesia, the beef was also found to be adulterated with rat [3]. This is because of the vast population of rats as pest in the paddy fields. Wild boar and rats are haram animals, which means they are strictly prohibited from being consumed by Muslim. This adulteration practice is not only important for halal-haram issue but also for ethical violation in general. Once being processed into meat-derived products such as

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meatball, these different types of meats are difficult to differentiate. Therefore, research providing information on the physical and chemical difference between different type of product made from different meats are highly required. The data then can be used as a basis of development of new authentication tool for meat products - adulteration detection.

Several techniques for meat authentication have been developed. For example molecular biology-based technique using enzyme-linked immunological methods [4] and DNA-based markers [5]. Other techniques include various spectroscopy and chromatography methods which targeting numerous primary and secondary metabolites present in different meat and meat processed products [6, 7, 8, 9, 10]. Metabolomics is one of emerging tool for such a purpose. The main technique in metabolomics is metabolic fingerprinting, which is a non-targeted technology that considers all detectable peaks or signals, including those from unknown analytes, for sample classification [11]. Metabolomics is viewed as potential tool to be applied for significantly reduce food fraud and its negative impacts [12]. Volatilomics is a metabolomics field that detects, identifies, and quantifies volatile metabolites in a biological system. Its contribution is highly important in several food areas, such as safety, quality, and authenticity [13]. GC is a suitable for identifying volatile compounds in meat and its processed product because each type of meat has a distinctive aroma related to its volatile components [14, 15]. SPME could be used to facilitate sample preparation on GC-MS instrumentation. SPME is widely applied in analytical practice because of its simplicity, solvent-free operation, short extraction time, and the possibility of automation. Also, the technique is favored due to its straightforward linkup with GC and relatively good results in the isolation of trace analytes [16, 17]. SPME technique coupled to GC–MS was reportedly as a powerful tool to differentiate various type of samples based on their volatiles profile differences, e.g. fresh raw beef quality with different lipid oxidation rate [18], four different pig breeds [14], and cooked beef with different period of aging [19], and meatballs made from beef, chicken and wild boar [10]. In addition to meat authentication based on volatiles profile, some studies used meat physicochemical properties for the detection of meat species and their processed products [20, 21, 22].

The objectives of the above-mentioned studies on SPME-GCMS-based volatile compounds and physicochemical property characterization of meat were mostly related to meat quality in general, with the effect of different processing methods, storage conditions, and species or breeds being studied. A similar method can be used to distinguish between meatballs made from halal and non-halal animals. This study determined the volatile compounds and physicochemical profile of meatballs made from halal (beef), and non-halal (rats and wild boar) animals, as well as their combinations at various compositions using SPME-GC/MS. The discriminating volatile compounds for each group of samples were determined using multivariate data analysis. PCA, an unsupervised feature of multivariate data analysis, was used as a first-pass method to identify differences in volatile compounds of meatballs [23]. The combined use of PCA and PLS-DA in data processing provide valuable insights into general spectral trends and predictive spectral features of the group of the meat type under study [24]. The classification pattern produced by PCA was then refined using PLS-DA [25]. Cross-validation and response permutation tests were then used to test the reliability of the resulted PCA and PLS-DA. Discriminating volatile compounds for each type of meatballs were selected based on the correlation coefficient and VIP values. Additionally, their physical properties which include texture and color, were also measured and analyzed using PCA to observe typical texture and color features for each type of meatballs.

2. Materials and methods

2.1. Materials

2.1.1. Raw meat sample collection

All the meat used in this research was purchased from the market. Samples of rats (*Rattus argentiventer*) were taken at random from a trader in Subang, West Java, Indonesia. Then, 400 female rats were selected with a bodyweight of 80–200 g. The rat meat was separated from the bones, then mixed, ground and homogenized. The wild boar (*Sus scrofa*) samples were selected from six female wild boars weighing 50–60 kg from the market in Banyu Asin Forest, South Sumatera, Indonesia. The frozen wild boars were wrapped in sack and transported to Bogor city. The ham, belly, and loin parts were taken from each wild boar in equal amounts, mixed and put in a sealed bag. The silverside of six *Brahman cross* cows (*Bos taurus*), weighing 400–550 kg, was obtained from the market in Bogor, West Java, Indonesia. The meat was purchased 36 h after the animals were slaughtered in halal slaughterhouse (RPH Bubulak, Bogor, West Java, Indonesia). The meat from different individual wild boars and cows was kept separate until they were processed into meatballs. Before use, all meat samples were kept in the freezer (−33 °C). The meat was thawed for 12 h before processed into meatballs. The meat was ground and homogenized before being processed into meatballs.

2.1.2. Meatball sample preparation

Meatballs are made according to a recipe that is usually made in Indonesia. The meatballs formulation used in this study was summarized in Table S1 (Supplementary Data). The meatball was prepared only using raw meat, tapioca 5% and ice cube 20%. No garlic, pepper, and sodium tri polyphosphate were used to avoid masking effects to the volatile profile of the samples. Meats were cut into small pieces (2 × 2 × 2 cm), then mixed and ground together with tapioca and ice cube. The dough was then rounded manually with diameter of 2.5 cm and weight 10 g, approximately. The raw meatballs were then put in boiling water for 10 min, then drained and cooled at room temperature for 15 min.

Pure beef and wild boar meatballs (MB, MW) were prepared in 3 separate batches using meat from different individual animal, except rat (MR). In case of mixed meatballs preparation, all meat from individual beef, or from indivudual wild boar, were mixed and homogenized. Meatballs made from a mixture of beef and rat at 4 ratios (2:8, 4:6, 6:4, and 8:2) were prepared separately as individual batches (MB2R8, MB4R6, MB6R4, MB8R2, respectively) with 2 replications each, as well as meatballs made from a mixture of beef and wild boar (MB2W8, MB4W6, MB6W4, MB8W2). All cooking utensils were carefully washed and drained before preparing the new batch. There were in total 25 batches, resulting in 250 meatballs. Twenty five meatballs were randomly selected for volatile analysis using SPME-GC/MS. For color and texture analysis, meatballs were prepared in similar way. The meatballs were made in 3 independent batches. Five meatballs were taken from each batch.

2.2. Methods of texture, color, and volatile analysis

2.2.1. Texture profile analysis

Meatballs were heated at 80 °C for 5 min and cooled at the room temperature. Then, they were cut off two sides to get 2 of 10 mm depth strips. The texture profile analyses (TPA) of meatballs were determined by using a texture analyser (Model TA-XT2 Texture Analysis, England) and equipped with a 25 kg load cell and the spherical probe (p/0.5 s, 1.2 cm diameter ball probe). The texture analyser's conditions were as follows: pre-test speed of 2.0 mm s^{-1}; post-test speed of 5.0 mm s^{-1}; test time 5.0 s; trigger type auto; and trigger force of 10 g. TPA measurements were done per strip for a total of five meatballs for each batch. The measurements were taken for hardness, springiness, cohesiveness, gumminess, and chewiness [26].

2.2.2. Color

Color values of meatball were determined using chromameter CR-400 (Minolta, Japan) set to the L*, a*, b* color space and illuminant D65, observer angle of 2°, aperture size of 10 mm. The instrument was calibrated using a white standard plate before color readings were performed. The color values were measured following exposure to air for 15

min (bloom time) after meatball surface were cut off two sides to get 2 of 10 mm depth strips. Three measurements were taken across the face of five meatballs for each batch.

2.2.3. SPME procedure

The volatiles were absorbed using a DVB/CAR/PDMS 2 ml fiber SPME apparatus (Supelco, Bellefonte, PA, USA). Before use, the fiber was heated in a GC-MS injector at 250 °C for 15 min to remove contaminants. Next, 8 g of minced meatball was added into a 22 ml glass vial with PTFE/Silicone septa (Agilent). The vial was closed hermetically, and the contents were put in a water bath for 80 min at 45 °C to extract volatile compounds, and the extracted fiber was injected into GC-MS. Desorption of volatile compounds occurs in the injection port of GC MS for 5 min. To remove volatile contaminants, the fiber was exposed to the GC injection port for 15 min before the analysis [10].

2.2.4. GC–MS protocol

An Agilent 7890A GC (Agilent Technologies, Santa Clara, USA) and an Agilent 5973C XL EI/CI MSD MS were used in this study. Helium gas was used as a carrier at a constant flow rate of 1 ml/min. The injection port was equipped with a 0.75 mm i.d, Agilent liner suitable for SPME. GC-MS analysis was conducted by inserting the fiber previously exposed to the samples into the injection port. The sample was injected in the spitless mode (250 °C). The compounds were separated in a capillary DB-WAX column with 30 × 0.25 mm dimensions and a film thickness of 0.25 μm (Agilent Technologies, Santa Clara, USA). The oven temperature was set at 40 °C for 5 min and then increased to 150 °C (4 °C min^{-1}). The temperature was further raised to 250 °C (30 °C min^{-1}), and held for 5 min. The interface temperature was maintained at 280 °C. The MS was operated in the electron ionization (70 eV), a scanning range of 29–550 *m/z*, a speed of 4.37 scans s^{-1}, and a gain factor of 1. The ion source and quadrupole analyzer temperatures were set at 230 °C and 150 °C, respectively [10, 27].

2.2.5. Statistical analysis and multivariate data analysis for texture and color measurement data

The data of texture and colour were analysed using PCA (SIMCA-P software v. 16.0, Sartorius-Umetric, Umea, Sweden). The data obtained from beef-rat and beef-wild boar meatballs at 40:50 ratios were also analysed using a nested design with the type of meatball as a fixed effect while the individual as a random effect. Duncan's new multiple range tests were also used to resolve the difference among treatment means. A value of $P < 0.05$ was used to indicate a significant difference.

2.2.6. Data processing and multivariate data analysis for volatile compounds

The Agilent GC–MS was used to process the collected raw data, including peak area integration and normalization. This process obtained a data matrix containing sample information and relative intensities of the compounds. GC–MS data was also manually annotated based on metabolites mass spectra comparisons between the Chemstation E. 02.02.1431 output and the NIST14 Mass Spectral Library. Each annotated metabolite's linear retention index (LRI) was calculated by comparing their retention time on the DB-WAX column to the retention time of the alkane solution (C8-40, Sigma Aldrich, Germany; 5 mg/L). The identified volatile compounds were input as raw data for PCA and PLS-DA (SIMCA-P software v. 16.0, Sartorius-Umetric, Umea, Sweden). Pareto scaling was used to remove noise caused by instrumentation error or other possible causes before the data was analyzed using multivariate data analysis. Cross-validation and response permutation tests were used to validate the PCA and PLS-DA models. The validation indicator represented by Q^2 values of at least 0.4 are considered acceptable. A credible model should have a higher Q^2 value in permutation testing than Q^2 values generated by random models utilizing the same data set [24]. Significant discriminating compounds for each group were selected based on the VIP and coefficient correlation value [10].

3. Results and discussion

3.1. Texture and colour profile of beef, rat, wild boar, and the mixture meatballs

3.1.1. PCA analysis

PCA with 4 components for texture and colour measurement data was first conducted with only meatballs made from pure beef, rat and wild boar (PCA1). The PCA explained 92.7% of total variation ($R^2X = 0.927$) with $Q^2 = 0.59$, indicating model reliability [24]. The score plot and loading plot of the first two components was shown in Figure 1A (i) and (ii), respectively. A clear grouping pattern between the three types of meatballs was observed. Next, PCA was also separately conducted for texture and colour measurement data of pure beef meatballs, pure rat meatballs, pure wild boar meatballs, and a mixture of beef-wild boar meatballs (PCA2) as can be seen in Figure 1B (i) and (ii) (five components, $R^2X = 0.936$ and $Q^2 = 0.483$). Lastly, PCA was conducted for pure beef meatballs, pure rat meatballs, pure wild boar meatballs, and a mixture of beef-rat meatballs (PCA3), presented in Figure 1C (i) and (ii) (three components $R^2X = 0.801$, and $Q^2 = 0.581$). In the PCA2 score plot of the first two components as shown in Figure 1B (i), meatballs made from a mixture of beef and wild boar at different compositions were scattered between pure beef and pure wild boar meatballs. Interestingly, their positions reflect the percentage of the meat type; for example, meatballs made up of 80% beef and 20% wild boar (MW2B8 at all replications) were closely clustered around pure beef meatballs, which were followed by their counterparts (MW4B6, MW6B4, and MW8B2). The last group, which made up of 80% wild boar and 20% beef (MW8B2), was the closest to the pure wild boar meatballs. A similar pattern was observed in PCA3 score plot (Figure 1C (i)), where meatballs made from the mixture of beef and rat at different compositions were scattered between pure beef and pure rat meatballs. In the loading part of all PCAs (as presented in Figure 1A (ii), B (ii), and C (ii)) a similar pattern of textural and colour features unique to each cluster was observed. Beef meatballs were characterized by high cohesiveness, hardness, chewiness, and gumminess, while wild boar and rat meatballs were the opposite. Wild boar had a typical high score of C, L, and b* values. Rat meatballs were characterized by a high redness (a*) score.

The texture and color of mixture meatballs are determined by the percentage of raw meat ingredients used. For example, meatballs made up of 20% wild boar and 80% beef were highly influenced by cohesiveness, hardness, chewiness, and gumminess, similar to those of pure meatballs. Only when meatballs were composed of at least 60% wild boar and 40% beef, did the texture and color of wild boar meatballs resembled pure wild boar meatballs. A similar pattern was observed in beef and rat meatballs. These patterns indicated that when wild boar or rat meats were used to adulterate beef in meatball products at a percentage less than 40%, the texture and color properties could not be distinguished from pure beef meatballs.

3.1.2. Two way ANOVA of texture and color data of MR6B4 and MW6B4

For mixture meatballs, those with ratios of beef and non-beef of 40:60 (MR6B4 and MW6B4) were chosen to be separately ananlyzed using two way ANOVA and Duncan's test (Table 1). Based on the interview with the trader in the market, this ratio was the most frequently used in adulteration practice. In their respective PCA biplots, these mixtures were located between their countermates made from pure meats. Separate statistical analysis between beef, rat, wild boar, MR6B4 and MW6B4 revealed that there was no consistent pattern in the measured texture features among different type of meatballs, as shown in Table 1. It is noteworthy that, except for cohesiveness, all texture features of MR6B4 and MW6B4 were significantly different as compared with pure beef meatballs ($P < 0.05$). For color analysis, only MW6B4 was significantly different in all color features as compared to beef meatballs. Rat meatballs and MR6B4 was significantly different

Figure 1. PCA of texture and colour measurement data: A. PCA1 score plot of pure beef, pure rat, and pure wild boar meatballs (i) and its loading plot (ii). B. PCA2 score plot of pure beef, pure rat, pure wild boar, and beef-wild boar mixture meatballs (i) and its loading plot (ii). C. PCA3 score plot of pure beef, pure rat, pure wild boar, and beef-rat mixture meatballs (i) and its loading (ii). The letters B, W, and R stand for pure beef, wild boar, and rat meatballs, respectively, while the number represents replication. M = mixture, the number next to the letter representing the percentage of each type of meat, while the last number in each sample code represents replication.

from beef meatballs and wild boar in L* value, indicating that meatballs made with rat as an adulterant might have the darkest color than those of beef and wild boar meatballs.

The results of the textural and color characterization of meatballs as mentioned in Table 1 supported the previous results that it could be useful to distinguish meatballs based on their raw meat materials only when the adulterant meat is used in relatively higher percentage (≥50%). This is in accordance with previous study which measured similar texture and color features of meatballs made from beef, rat, pork, dog, and their mixtures [28]. Currently, there are not so many studies reporting the physical characteristics of meatballs made of different types of meat.

3.2. Volatiles profile of meatball samples

Overall, 404 volatile compounds were identified in beef meatballs, 371 in rat meatballs, 283 in wild boar meatballs, 956 in meatballs made from beef and rat mixture, and 885 in meatballs made from beef and wild boar mixture (Table 2).

Table 1. The texture and color parameters of beef meatballs, rat meatballs, wild boar meatballs, and their mixtures.

Parameter	MB	MR	MW	MR6B4	MW6B4
Texture					
Hardness	1334.59 ± 38.01^{c}	840,39 ± 44.36^{a}	867,24 ± 44.46^{a}	979.01 ± 45.57^{b}	1019.06 ± 29.65^{b}
Cohesiveness	0.65 ± 0.00^{a}	0.68 ± 0.06^{a}	0.64 ± 0.00^{a}	0.62 ± 0.01^{a}	0.65 ± 0.01^{a}
Gumminess	859.78 ± 22.67^{c}	575.62 ± 69.38^{ab}	552.91 ± 26.42^{a}	614.87 ± 32.25^{ab}	662.57 ± 17.78^{b}
Chewiness	859.78 ± 22.67^{c}	575.83 ± 69.31^{ab}	552.91 ± 26.42^{a}	614.87 ± 32.26^{ab}	662.57 ± 17.78^{b}
Color					
Lightness (L*)	65.55 ± 0.68^{c}	55.16 ± 0.55^{a}	64.11 ± 0.55^{cd}	60.99 ± 0.63^{b}	64.77.99 ± 0.95^{d}
Redness (a*)	3.59 ± 0.17^{a}	4.43 ± 0.15^{c}	5.05 ± 0.10^{d}	3.80 ± 0.18^{ab}	4.03 ± 0.12^{b}
Yellowness (b*)	14.73 ± 0.20^{a}	14.95 ± 0.27^{a}	16.02 ± 0.21^{b}	15.15 ± 0.18^{a}	15.81 ± 0.16^{b}

* Data are means ± SE. Mean values in the same row that are followed by same letters are not significantly different ($P > 0.05$).

Distribution of volatile compounds present in the meatballs samples was summarized in Figure 2. Alcohols, aldehydes, and aromatic hydrocarbons were the major volatiles found in all type of meatballs, whereas carboxylic acid, ester, ether, nitrogen- and sulphuric-compounds were detected in lower amounts. Hundreds of volatile compounds found in cooked meat include lipid and fatty acid oxidation products such as aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids, and esters [29].

It is obvious that meatballs made from a meat mixture had the highest total volatile compounds compared to those of meatballs made from single meat. Many factors are known to influence meat flavor, including an animal's breed, sex, food, and age; the conditions and procedure of slaughter; the duration and conditions of meat storage; the type of muscle; the preparation of meat and the type of additives used, as well as the heat treatment condition (cooking, roasting, smoking) [29]. The most important influences, however, are most likely genetic and environmental [15]. Raw meat has a very faint odor, indicating a lack of volatiles, which contribute to the distinctive meaty aroma. However, raw meat contains a significant amount of nonvolatile chemicals, which act as precursors to volatiles responsible for the flavor of diverse meat products. Amino acids, peptides, saccharides, inorganic salts, and inorganic acid are among the precursors, with amino acids, peptides, and reducing sugars being the most important [29]. It was recently reported that different fresh meats, such as beef, pork, lamb, chicken, and turkey, contained qualitatively and quantitatively different types of amino acids [30]. For example, serine was only identified in pork leg, turkey leg, and chicken breast, but not in lamb and beef legs. Fat and fatty acids are also particularly important in species-specific flavor imparting volatiles formation. A previous report showed that different animal species had different fatty acids profile [31]. Lauric acid, for example, is found in beef and pork but not in lamb or chicken. Some species may contain the same amino acids or fatty acids in different concentrations. Meat thermal processing produces a large number of volatiles as a result of various reactions such as Maillard reactions, lipid oxidation, interactions between Maillard reaction products and lipid oxidation, Strecker degradation, and carbohydrate breakdown [29, 32]. It is understandable that the resulting volatile compounds will differ when the precursors used in these reactions differ qualitatively and quantitatively. In other words, when meat from different species are mixed and heated, the volatiles produced are more diverse than those produced by single meat because more diverse precursors involve in their formation. The data presented above can explain why meatballs made from a meat mixture contained more volatiles than their single counterparts, as found in our study (Figure 2). These findings are consistent with recent research by Leng et al. (2020), who discovered that the total volatile basic nitrogen (TVB-N) content of a mixture of minced beef and pork was significantly higher than the TVB-N content of minced beef alone or minced pork alone [33].

3.3. PCA of volatile data of all meatball samples

Unsupervised PCA was then used to study the meatballs classification pattern based on the volatile compound composition. Exponentially Weighted Moving Average (EWMA) filtering was applied to remove the signal noise. The resulted PCA explained 82.7% of total variation (R^2X 0.827) and Q^2 0.661, indicating the reliability of the model [24]. The PCA 3D score plot of the first three components showed that beef meatballs (MB, green bullets), rat meatballs (MR, blue bullets), and wild boar meatballs (MW, black bullets) were well-separated (Figure 3). The meatballs made from a mixture of beef and rat (MBR, red bullet) and a mixture of beef and wild boar (MBW, yellow bullets) were scattered between the beef meatballs, the rat meatballs, and wild boar meatballs. These patterns indicated that the data can be further analyzed using a supervised multivariate data analysis PLS-DA to fine tune the discriminating volatiles for each group.

3.4. PLS-DA of meatballs made from pure beef, rat, wild boar meatballs, and their combinations

In this study, beef meatball adulteration was simulated by combining beef with wild boar or rat meat. Because there has been no report of beef meatballs being contaminated with rat and wild boar meat, this mixture was not used in this study. The discriminating compounds in various types of meatballs were determined using PLS-DA. The analysis was divided into two steps to obtain a clearer classification. PLS-DA was first performed on pure beef meatballs, and pure rat meatballs (Figure 4).

Next, a different PLS-DA model was created for pure beef meatballs, pure wild boar meatballs, and meatballs from a mixture thereof (Figure 5). Each PLS-DA score plot showed a distinct clustering between different groups of samples (Figures 4 and 5).

Validation with 100 random permutations was performed to assess the reliability of the PLS-DA model constructed from the volatile data of pure beef meatballs, pure rat meatballs, and meatballs made from their mixtures (Supplementary Figure S1). R^2Y and Q^2Y values (green circles and blue squares in the bottom-left corner) of the permuted models were lower than the associated initial values (green circles and blue squares in the top-right corner of the plot). This indicates the model's stability and reliability [34]. Additionally, the p-value for the cross-validated analysis of variance (CV-ANOVA) was 0.000370626, less than 0.005, demonstrating good model validity [35]. Similar permutation test and CV-ANOVA were also used to validate the PLS-DA model created from volatile data of pure beef meatballs, pure wild boar meatballs, and meatballs made from their mixture (Supplementary Figure S2). All the validation data indicated a good reliability of the PLS-DA model. Selection of volatile marker compounds for each PLS-DA class were done based on the coefficient correlation and VIP value. Only volatile compounds with positive coefficient correlation and the VIP values >1 were selected from PLS-DA of meatballs volatiles data.

Table 2. Volatile compounds identified in beef, rat, wild boar meatball and their mixtures using SPME/GC MS.

Compound	RT	LRI	Method[a]	Peak Area (x104)				
				B	R	WB	B/R	B/WB
Aldehydes								
Butanal, 3-methyl-	3.6909	914	L	829.36	1251.58	-	-	70.93
Pentanal	3.7026	935	L	2210.06	9324.84	3183.72	5054.53	5720.46
Glutaraldehyde	6.1880	1072	M	2066.05	1396.36	258.84	2349.70	23.41
Hexanal	6.3545	1076	L	25117.44	70275.80	46297.69	55838.56	72894.84
Heptanal	9.6426	1174	L	5208.07	4705.72	1021.62	7205.03	7389.87
n-Octanal	13.5789	1280	L	311.48	-	276.15	937.56	1450.14
2-Heptenal, (Z)-	14.7681	1320	L	272.03	17027.49	175.46	1510.37	2424.62
Nonanal	17.1942	1368	L	1041.78	6432.23	1860.64	12919.27	15511.35
2-Octenal, (E)-	18.4489	1408	L	37.71	517.14	132.47	189.79	386.69
2,4-Heptadien-1-al	20.3454	1478	L	98.47	248.17	18.47	266.86	91.99
Decanal	20.8568	1494	L	492.15	951.21	687.76	629.96	751.03
Benzaldehyde	21.1362	1513	L	3100.53	1476.65	236.70	2635.20	1625.47
2-Nonenal, (E)-	21.5346	1509	L	249.77	328.47	-	313.61	8.93
2-Nonenal	21.8081	1532	L	38.63	279.99	-	17.37	-
4-Ethylbenzaldehyde	25.6255	1650	M	20.36	34.04	11.62	49.46	10.92
2-Octenal, 2-butyl-	26.0655	1665	M	507.18	231.19	266.97	23.51	428.48
Benzaldehyde, 3-ethyl-	26.7198	1688	M	1045.83	1596.17	424.86	1232.81	604.98
Tetradec-2-enal, (E)-	28.1644	1740	M	87.96	-	-	203.35	10.32
2-Dodecenal, (E)-	28.2895	1744	M	34.77	23.52	50.01	127.88	184.88
2-Undecenal, (E)-	28.3249	1748	L	53.52	8.91	8.47	112.99	138.14
3-Methyl-2-thiophenecarboxaldehyde	28.9076	1813	L	3.86	-	60.92	63.74	41.52
Tetradecanal	32.8676	1927	L	331.89	153.16	-	262.10	231.92
Benzaldehyde, 4-pentyl-	33.9201	1997	M	298.15	248.99	106.63	363.43	242.49
Heptadecanal	34.0211	2005	M	265.79	752.35	46.52	640.47	62.83
17-Octadecenal	34.2055	2025	M	123.68	65.87	19.15	255.60	75.04
Octadecanal	34.9606	2108	M	138.26	39.42	252.67	431.15	191.35
2,4-Decadienal	28.7054	1777	L	8.12	49.81	18.46	38.73	20.94
2-Tridecenal, (E)-	21.7309	1520	M	-	690.85	334.92	125.30	573.37
2-Decenal, (E)-	25.2270	1640	L	-	194.52	113.99	120.76	148.27
2-Undecenal	27.8849	1712	L	-	36.16	-	192.57	-
2,4-Decadienal, (E, E)-	29.8771	1808	L	-	-	26.91	156.54	141.32
Alkanes								
5-Ethyl-2-methyloctane	5.6234	1053	M	1336.53	1115.35	613.19	690.41	628.86
Undecane, 5,7-dimethyl-	5.9861	1065	M	386.79	-	-	-	-
Nonane, 3-methyl-	6.8899	1095	M	886.24	-	-	-	-
Undecane, 3-methyl-	7.1039	1101	M	840.20	-	2591.05	-	-
2,4-Dimethylhexane	7.2228	1105	M	537.14	-	116.10	528.95	-
Undecane, 5-methyl-	8.1086	1128	M	1044.21	1414.79	276.66	969.28	608.99
Undecane, 3,4-dimethyl-	8.3405	1135	M	549.18	824.11	270.42	514.74	316.45
3,5-Dimethylheptane	10.5110	1193	M	56.41	188.51	-	-	96.19
2,4,6-Trimethyloctane	10.6299	1197	M	188.68	239.00	265.15	-	896.76
2-Methyltridecane	11.1769	1211	M	140.72	274.62	-	-	9.51
3,6-Dimethylundecane	11.9617	1233	M	327.33	968.13	-	-	246.00
3,7-Dimethylnonane	12.7170	1254	M	52.02	253.19	84.14	13.88	113.94
Undecane,4,7-dimethyl-	13.3946	1272	M	280.04	1314.47	141.64	1342.78	789.21
Undecane,3,7-dimethyl-	16.0586	1348	M	279.34	885.84	-	207.43	467.20
Tetradecane	17.8184	1398	L	149.89	44.51	52.51	322.02	518.38
2-Methyldecane	19.7687	1457	M	156.48	-	1180.44	629.51	-
Pentadecane	20.9046	1497	L	24.92	-	-	186.42	-
Hexadecane	24.0560	1596	L	192.28	156.33	173.11	172.96	183.91
Cyclopropane, nonyl	25.8514	1658	M	84.49	50.84	3.89	83.61	27.58
Isopropylcyclohexane	22.6584	1550	M	22.27	275.66	300.61	197.75	176.85
3,8-Dimethyldecane	11.3493	1215	M	46.78	279.01	-	-	117.02
Tridecane	11.1769	1211	M	140.72	274.62	-	-	9.51
Cyclopentane, nonyl-	19.4299	1448	M	-	-	318.17	39.67	158.41
Dodecane	9.9461	1198	L	-	-	402.86	-	-
Cyclooctane, methyl-	14.2034	1294	M	-	-	-	40.27	143.42

(*continued on next page*)

Table 2 (*continued*)

Compound	RT	LRI	Method[a]	Peak Area (x104)				
				B	R	WB	B/R	B/WB
Alkenes								
6-Dodecene, (E)-	9.0660	1154	M	118.14	257.51	-	50.35	208.99
Cyclopentene, 1-ethenyl-3-methylene-	9.2562	1159	M	569.74	1099.74	259.04	945.76	307.25
4-Ethylcyclohexene	11.0400	1208	M	181.28	156.59	70.16	47.05	105.58
3-Ethyl-2-methyl-1,3-hexadiene	17.4497	1388	M	369.00	402.71	82.80	359.55	29.55
1-Octene, 3,7-dimethyl-	17.6519	1394	M	159.67	278.74	37.55	690.85	1744.19
1-Tetradecene	18.7697	1450	L	76.79	-	21.31	37.31	128.29
1-Hexene, 3,5,5-trimethyl-	20.6309	1483	M	119.68	112.14	-	149.38	36.98
2-Undecene, 8-methyl-, (Z)-	22.3732	1541	M	1076.22	601.37	-	270.08	140.39
1,3-Hexadiene, 3-ethyl-2-methyl-, (Z)-	17.7411	1396	M	52.40	851.07	-	99.76	-
3,5-Dimethyl-1-hexene	23.0508	1563	M	-	694.98	-	222.55	-
Methyl ethyl cyclopentene	28.5925	1755	M	-	41.82	6.40	5.03	53.73
1-Decene, 3,4-dimethyl-	29.1457	1775	M	-	-	15.84	19.15	25.51
Azulene	27.6550	1721	M	-	108.61	-	129.92	110.68
Alcohols								
Cyclobutanol	1.9961	<1000	M	1741.74	1437.29	12190.67	3462.21	8046.57
2-Ethylbutanol	7.5913	1115	M	866.11	563.05	399.20	247.56	368.55
2-Pentanol	7.6745	1120	L	1023.16	1273.37	385.66	1084.26	389.29
2-Butanol, 3-methyl-	8.2454	1107	L	359.58	502.84	194.74	388.73	407.82
2-Pentanol, 4-methyl-	11.6227	1124	L	168.13	405.50	120.10	117.45	409.42
3-Methyl-3-butenol	12.9427	1251	L	961.82	10463.23	877.73	155.48	329.16
1-Pentanol	13.0203	1257	L	2677.67	2233.80	410.10	6953.87	8196.79
6-Methyl-2-heptanol	14.4886	1302	M	220.62	8713.08	24.28	4293.39	1056.58
1-Undecanol	15.2973	1326	M	74.30	577.98	4152.22	286.56	2647.27
1-Hexanol	16.8373	1371	L	290.53	238.23	933.01	359.74	119.25
2-Butoxyethanol	18.0148	1402	L	387.20	761.47	-	968.10	317.32
2-Hepten-1-ol, (E)-	18.1575	1410	M	149.07	1039.53	62.12	274.58	411.73
Ethanol, 2-(dodecyloxy)-	19.2692	1443	M	93.08	230.07	24.00	208.28	223.50
1-Octen-3-ol	19.5308	1452	L	1998.06	9242.54	2088.50	7612.31	6004.95
1-Heptanol	19.8400	1460	L	550.28	742.95	60.89	1192.02	1594.27
5-Hepten-2-ol, 6-methyl-	20.0778	1464	L	762.42	465.73	193.73	371.99	44.30
7-Octen-2-ol, 2,6-dimethyl-	20.1968	1473	L	29.55	228.64	53.90	464.65	61.89
1-Octanol	19.3408	1445	M	-	179.34	50.81	50.58	60.77
Cyclooctanol	24.4541	1610	M	84.80	142.61	52.23	158.03	183.06
2-Octen-1-ol, (E)-	24.5552	1610	L	179.83	35.51	32.37	428.17	175.83
2-Nonen-1-ol, (E)-	24.7157	1691	L	169.05	1289.62	400.46	986.28	678.82
1-Dodecanol	33.6941	1978	L	504.40	224.90	117.01	399.17	160.61
3-Methylbutanol	11.3873	1212	L	145.76	-	-	147.32	-
2-Methyl-1-indanol	25.4292	1643	M	6.50	-	-	23.26	5.55
1-Octanol, 2-butyl-	19.3408	1445	M	-	179.34	50.81	50.58	60.77
Cyclohexanol, 2-tert-butyl-	22.0222	1529	M	-	3350.81	-	167.68	909.70
6-Methyl-1-octanol	23.4140	1575	M	-	150.77	11.22	329.78	52.53
Benzyl alcohol	31.7260	1865	L	-	341.57	698.08	411.11	975.51
5-Hexen-2-ol	29.3773	1784	M	-	-	8.31	19.30	-
2-Cyclohexen-1-ol	21.3860	1509	M	-	-	-	245.94	124.37
2-Butyl-2,7-octadien-1-ol	25.5482	1647	M	-	-	-	49.20	-
1-Nonanol	25.9406	1664	L	-	-	-	36.79	24.43
1-Heptanol, 2,4-dimethyl-,	14.5390	1304	M	-	-	-	2010.42	-
Carboxylic acids								
3-Hydroxybutyric acid	8.8935	1150	M	380.25	424.50	79.66	360.41	75.25
2-Methyldecanoic acid	23.2173	1569	M	89.62	31.30	-	213.90	-
2-Amino-6-methyl benzoic acid	30.0017	1807	M	9686.90	4607.38	1837.35	6314.18	2870.20
2-Amino-5-methyl benzoic acid	30.4060	1822	M	8434.68	3812.24	292.22	3281.03	1617.34
Caproic acid	31.0186	1829	L	-	2869.94	-	-	1406.97
Lauric acid	36.6733	2487	L	-	-	1597.67	-	2677.84
Acetic acid	19.5192	1450	L	-	-	-	41.13	-

(*continued on next page*)

Table 2 (*continued*)

Compound	RT	LRI	Method[a]	Peak Area (x104)				
				B	R	WB	B/R	B/WB
Esters								
Methyl caprylate	16.3020	1372	L	106.40	211.34	177.26	456.55	791.22
Methyl salicylate	28.4260	1745	L	5.56	51.40	-	53.68	2.36
Methyl palmitate	35.4838	2204	L	13.33	117.52	303.02	244.19	603.42
Methyl caprate	23.6812	1590	L	-	326.20	-	83.82	-
Eter								
2-Ethoxyethyl ether	18.3714	1415	M	69.80	-	-	267.29	500.63
Heterocyclics								
2-Methylthiophene	7.8767	1123	L	344.05	1236.38	349.47	1243.50	746.59
Furan, 2-pentyl-	10.7843	1215	L	1209.21	2509.36	748.20	2387.80	2286.41
Thiophene, 2-pentyl-	19.0138	1440	L	223.46	132.32	-	147.30	164.45
Acridine, 9-methyl-	21.5703	1515	M	1052.08	508.65	347.60	1279.47	298.88
Benzothiazole	33.2363	1961	L	709.72	221.55	70.31	263.41	128.33
3-Methyl-2-formylthiophene	35.1620	2144	M	68.93	95.56	-	4.64	48.31
Indole	36.5838	2441	L	1738.84	1399.66	1559.76	3432.41	3997.81
5-Methyl-2-phenylindole	34.5088	2057	M	46.23	38.62	44.59	6.79	53.13
Thiophene, 2-ethyl-5-isopentyl-	35.2819	2165	M	10.99	-	-	157.36	7.02
Thiophene, 2-butyl-5-ethyl-	34.3601	2041	M	-	-	14.38	81.03	17.31
Aromatic Hydrocarbons								
Ethylbenzene	7.4427	1119	L	2750.83	3388.71	959.96	2836.26	1569.30
p-Xylene	8.6021	1142	L	603.52	815.31	195.50	592.55	487.48
β-Cymene	11.8308	1277	L	432.47	327.82	117.42	523.64	287.84
Styrene	11.9735	1241	L	67.12	-	210.00	444.53	277.74
Benzene, 1,3,5-trimethyl-	12.4254	1246	L	34.27	1785.54	93.53	1983.99	244.20
Benzene, 1,2,3-trimethyl-	12.8000	1257	L	208.29	78.15	588.16	570.19	663.40
o-Cymene	13.9357	1287	L	201.96	477.38	-	93.18	603.36
m-Xylene, 5-ethyl	14.3400	1298	M	3117.24	-	126.80	972.60	119.84
Benzene, 2-propenyl-	15.8740	1342	M	288.92	159.07	112.10	382.15	245.30
Benzene, 1,2,4,5-tetramethyl-	17.9611	1419	L	245.58	75.75	869.91	369.15	428.05
o-Cymenene	18.5676	1421	M	102.01	186.50	175.75	227.07	410.97
Benzene, 1,2,3,5-tetramethyl-	18.8530	1430	L	20.42	153.46	407.61	191.07	104.91
Butylated Hydroxytoluene	32.6001	1899	L	168.83	99.27	-	101.47	17.81
Phenol, 3,5-dimethoxy-	33.0638	1941	M	200.31	116.29	24.90	207.12	60.84
m-Ethylphenol	34.4433	2050	M	58.87	35.82	138.76	64.65	32.36
o-Xylenol	34.5684	2063	M	50.65	124.23	5.89	83.76	37.84
p-Cresol	34.6217	2067	L	32.64	-	114.36	142.92	31.73
m-Cresol	34.8479	2115	L	71.31	542.59	88.23	46.11	379.40
cis-Isoeugenol	35.1987	2186	L	36.24	-	201.82	25.37	108.01
p-Vinylguaiacol	35.5730	2220	L	203.44	581.43	454.04	344.79	474.58
2,4-Di-tert-butyl-phenol	35.9001	2312	L	728.64	434.45	494.26	559.61	1047.35
p-Cymenene	18.6865	1437	L	71.01	247.75	199.06	260.16	313.52
m-Xylene	8.7211	1147	L	-		234.93	14.48	78.38
.psi.-Cumene	14.6016	1289	L	-	-	240.51	428.10	201.52
o-Xylene	10.2077	1183	L	-	-	163.72	-	-
2-Ethylnitrobenzene	26.8625	1693	M	-	-	-	738.49	35.22
Phenol, 2,4-dichloro-	35.2370	2157	M	-	-	-	14.56	-
Cardene	12.0509	1235	M	-	-	-	42.35	65.64
1,3-Dicyanobenzene	27.3559	1709	M	-	-	-	69.57	95.02
Naphthalene	27.5878	1718	L	155.27	1548.79	549.89	1865.57	644.39
Ketones								
6-Dodecanone	11.2481	1213	M	67.39	-	424.09	259.07	433.36
Cyclobutanone, 2,2-dimethyl-	11.5454	1222	M	180.59	59.51	217.88	338.79	71.07
2-Heptanone, 6-methyl-	12.2113	1240	L	227.91	300.79	246.47	256.03	384.99
3-Octanone	12.3839	1244	L	303.13	-	-	-	73.70
Acetoin	14.1200	1287	L	129.10	436.54	321.34	353.67	411.81
2,5-Octanedione	15.4284	1329	M	138.31	533.48	98.06	265.64	337.99
2,3-Octanedione	15.8029	1344	L	1786.77	246.97	114.60	915.70	825.61
2-Decanone	20.7914	1493	L	205.60	83.69	63.69	312.47	270.10
6,7-Dodecanedione	21.8973	1525	M	136.57	245.10	194.90	454.05	393.67

(*continued on next page*)

Table 2 (*continued*)

Compound	RT	LRI	Method[a]	Peak Area (x104)				
				B	R	WB	B/R	B/WB
Acetophenone	25.0427	1627	L	84.74	254.58	24.60	257.42	135.60
3-Tridecanone	29.0265	1797	L	12.12	142.29	18.69	208.37	65.31
2-Nonanone	17.2596	1375	L	331.88	747.17	-	543.74	20.67
11-Dodecen-2-one	23.7882	1587	M	27.04	66.15	65.32	51.19	78.54
γ-Nonalactone	34.1403	2026	L	50.03	44.87	44.04	23.55	132.11
Nona-3,5-dien-2-one	32.0411	1885	M	31.81	542.32	-	245.73	202.31
2-Undecanone	23.8597	1593	L	-	134.67	-	17.66	39.95
2-Methyl-3-octanone	15.1189	1322	L	-	355.34	239.85	294.43	267.15
5-Hepten-2-one, 6-methyl-	15.5292	1332	L	-	191.23	128.78	184.62	232.52
2,6-Dimethylcyclohexanone	16.3797	1322	L	-	96.03	-	529.02	328.85
3,5-Octadien-2-one	21.2849	1500	L	-	46.43	-	358.36	-
Tetrahydrothiopyran-4-one	33.9738	2000	M	-	-	1.60	142.78	326.32
4-Nonanone	21.9688	1528	M	-	-	-	-	47.75
Nitrogen compounds								
1,2,4-Triazol-4-amine, 5-ethyl-3-(3-methyl-5-phenylpyrazol-1-yl)-	22.5216	1546	M	243.01	392.52	584.08	495.45	730.46
Benzyl nitrile	32.7309	1918	L	108.25	146.33	-	220.32	20.25
Diethyltoluamide	35.8406	2278	M	-	72.48	475.31	219.65	31.70
Sulfur compounds								
Disulfide, dimethyl	6.0812	1071	L	1061.06	1533.49	439.25	1270.85	897.32
Disulfide, di-tert-dodecyl	14.9645	1316	M	181.91	-	-	-	60.44
Dimethyl trisulfide	15.0297	1329	L	210.67	198.81	746.64	2532.62	6007.90
Terpenoids								
Limonene	9.4524	1166	L	743.87	493.53	712.20	660.02	1287.35
α-Terpinolene	13.8524	1282	L	660.16	638.50	597.36	862.62	187.97
1,3,8-p-Menthatriene	19.9827	1411	L	119.00	-	-	540.66	-
d-2-Bornanone	20.7379	1491	L	113.38	948.28	61.85	334.72	489.97
Fenchol	23.5681	1574	L	154.38	235.87	20.85	362.13	14.46
Terpinen-4-ol	23.8833	1591	L	53.22	48.43	31.73	173.08	184.92
3-p-Menthol	25.1141	1612	L	104.33	147.46	45.52	169.79	-
Isoborneol	25.7444	1659	L	9.10	108.75	14.81	103.88	76.87
1-Terpinenol	23.3481	1573	L	-	279.60	49.66	140.56	-
Camphor	20.7676	1491	L	-	73.46	-	-	-
β-Terpineol	24.9060	1646	L	-	49.50	48.54	315.59	293.23
dl-Menthol	25.3344	1630	L	-	-	48.98	47.32	-
L-Camphor	21.0176	1511	L	-	-	45.83	67.15	40.72
α-Curcumene	28.6282	1773	L	-	-	-	135.76	23.81

[a] Reliability of identification (L: MS data and RI in agreement with those of authentic compounds; M: MS data in close agreement with the NIST14 Mass Spectral Library.

Cooked meat such as meatball contains a complex mixture of volatile compounds which may derived from lipid and water-soluble precursors. These compounds provide roast, boiled, fatty, species-related flavors and the characteristic aroma of cooked meats [36]. Moreover, lipid thermal degradation produces chemicals that give cooked meat its fatty odors and those that determine the flavors of various species [37]. However, many volatiles found in meat species may have contradictory results in the literature. Several compounds might be transmitted directly from ingested feeds into animal tissue, while others resulted from alteration of feed molecules by ruminal bacteria [27].

Table 3 summarizes the ten most significant volatile compounds positively associated with pure beef, pure rat, and beef-rat mixture meatballs. The complete list of volatiles with their coefficient and VIP value is available as supplementary data (Table S2). It is shown that 2-amino-5-methyl benzoic acid was identified as the most robust discriminator in the pure beef meatball class. Other volatile compounds markers for this group were 2-amino-6-methyl benzoic acid, heptanal, benzaldehyde, 5-ethyl-m-xylene, 2,3-octanedione, ethylbenzene (Z)-8-methyl-2-undecene, 5-ethyl-2-methyloctane, and 3-methyl-undecane. A previous study found that benzaldehyde, heptanal, and undecane were present in cooked beef [38]. Additionally, benzaldehyde, heptanal, 2,5-cotanedione, and undecane were also identified in roasted beef [39].

The most robust discriminator in the rat meatball class was (Z)-2-heptenal, followed by 3-methyl-3-butenol, caproic acid, pentanal, 2-tert-butyl-cyclohexanol, 3-methyl- butanal, 6-methyl-2-heptanol, and 3,6-dimethylundecane. Nonanal was the most significant discriminator in the beef and rat meatball mixture. Other volatile markers for this class also included 1-pentanol, cyclobutanol, 1-octen-3-ol, 1-octanol, indole, dimethyl trisulfide, benzene, 1,3,5-trimethyl-, 2-ethylnitrobenzene, naphthalene. A previous study found benzaldehyde, 1-octen-3-ol, hexanal dimethyl trisulphide in cooked beef [15]. In this previous report, 1-octen-3-ol is an alcohol group compound with a mildew-like odor found in fresh meat stew. It has an important role in the stew's flavor [15]. Recent studies used FTIR spectroscopy and multivariate data analysis to detect rat adulteration in raw beef and beef meatballs based on typical functional group profiles [3, 40]. The study successfully

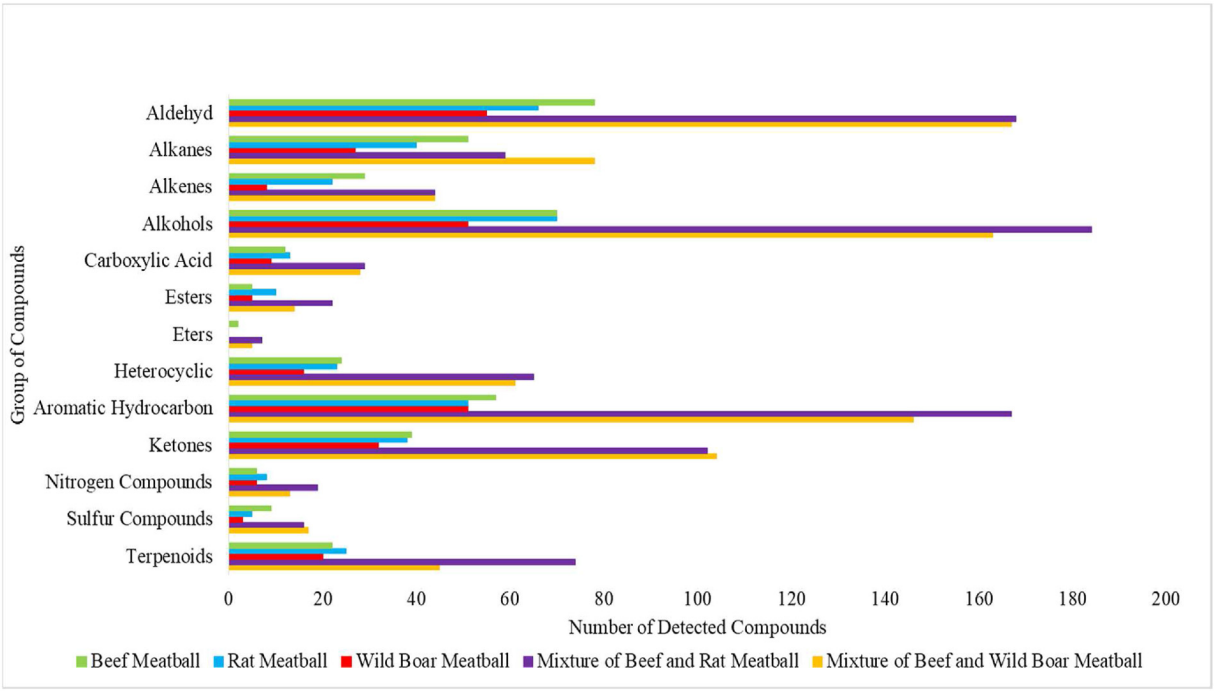

Figure 2. The distribution of the volatile compounds detected in each type of meatball.

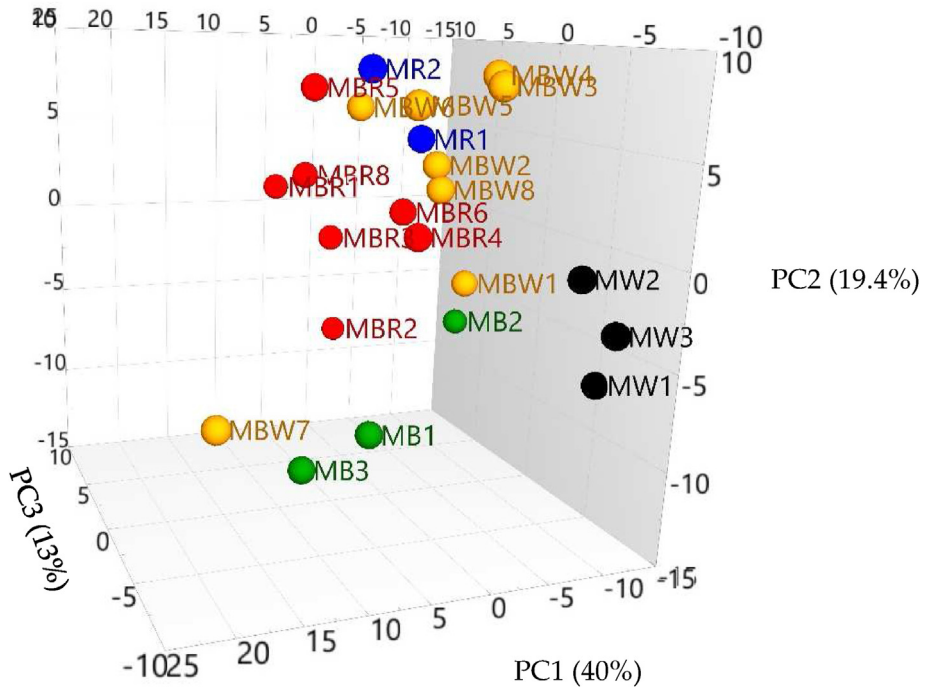

Figure 3. The first 3 components of PCA score plot of volatiles data: pure beef meatballs (M, green), pure rat meatballs (MR, blue), pure wild boar meatballs (MW, wild boar), meatballs made from the mixture of beef-rat (MBR, red), and meatballs made from the mixture of beef-wild boar (MBW, yellow).

classify rat's meatball and beef meatballs including samples obtained from the market [3], and successfully classify lipid components extracted from beef meatballs and rat's meatballs using three different lipid extraction methods with 100% accuracy [40]. However, because studies on volatile compound characterization in rat raw meat or rat meatballs are uncommon, it is difficult to compare the volatiles data obtained in the present study with other reports.

The ten most significant positive discriminating compounds in PLS-DA of pure beef meatballs, pure wild boar meatballs, and meatballs made from their mixtures were summarized in Table 4. The complete list of volatiles with their coefficient and VIP value is available as supplementary data (Table S3). The beef discriminating volatile profile in this PLS-DA was nearly identical to that of the PLS-DA of beef, rats, and beef-rat mixture meatballs shown in Table 3, except that 6-methyl-5-hepten-2-ol and glutaraldehyde were not identified as significant markers here. Cyclobutanol was identified as the strongest discriminator in the wild boar meatballs class. Other volatile compounds were also identified which included undecane, 3-methyl-, 2-methyl decane, 1-hexanol, lauric

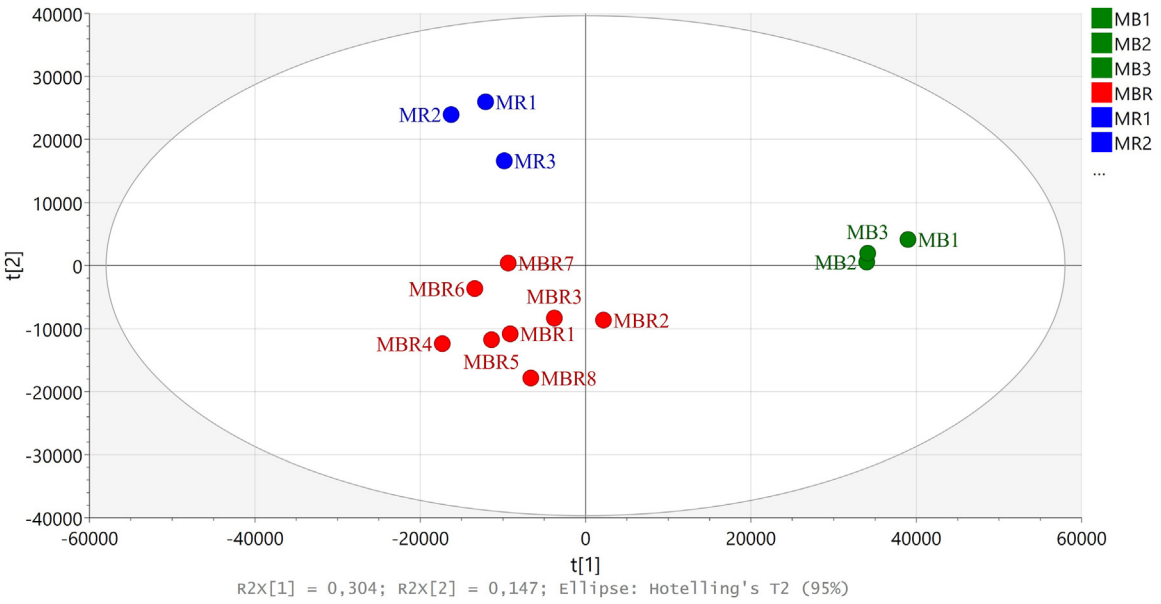

Figure 4. The first two components of PLS-DA score plot of pure beef meatballs (MB, green), pure rat meatballs (MR, blue), and beef-rat mixture meatballs (MBR, red) volatile data (R^2X 0.451, R^2Y 0.915 and Q^2 0.729).

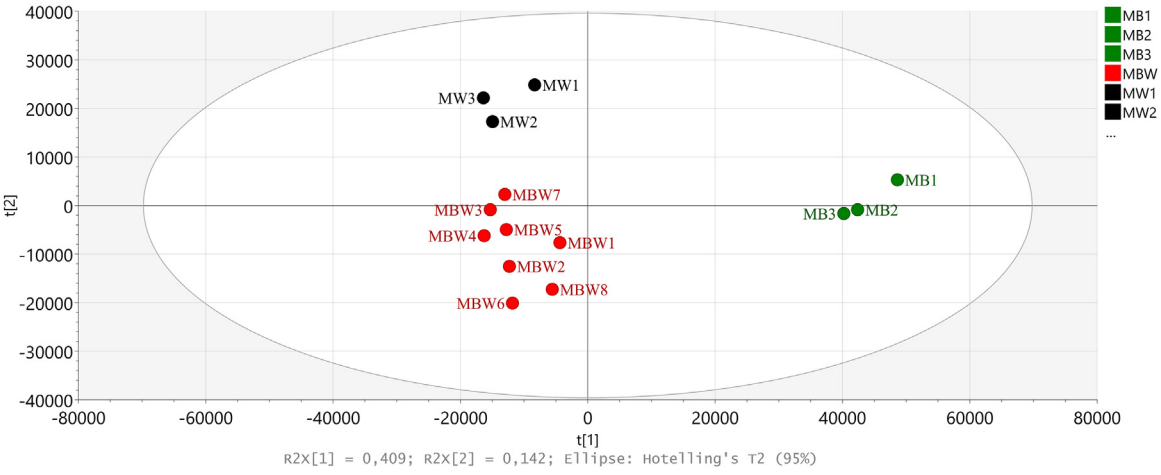

Figure 5. The first two components of PLS-DA score plot of pure beef meatballs (MB, green), pure wild boar meatball (MW, black), and beef-wild boar mixture meatballs (MBW, red) volatiles data (R^2X 0.763, R^2Y 0.991, Q^2 0.883).

acid, benzene, 1,2,4,5-tetramethyl-, and diethyltoluamide. A previous study reported that benzaldehyde, heptanal, 2,5-octanedione, and undecane were detected in roasted pork [39]. Hexanal compounds were also discovered in pork cheeks cooked at various temperatures [41].

The strongest discriminator in the mixture of beef and wild boar meatball was nonanal. Other markers included 1-pentanol, heptanal, 1-octene, 3,7-dimethyl-, dimethyl trisulfide, 1-heptanol, caproic acid, and 2,4,6-trimethylolethane. It was reported that key volatiles of cooked beef and pork were as follow; octanal, nonanal (E,E)-2,4-decadienal, methanethiol, methional, 2-furfurylthiol, 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, and 4-hydroxy-2,5-dimethyl-3-(2H)-furanone [29]. A previous study also showed that nonanal, heptanal, 1-pentanol and 1-heptanol, and dimethyl trisulfide were the volatile compounds in boiled pork [42] and in cooked beef [38]. There are currently few reports on volatile compounds in wild boar meatballs [10], whereas the presence of volatiles in fried wild boar meat was recently summarized [1, 43]. Three significant markers for wild boar were in agreement with previous report, those are cyclobutanol, lauric acid, and 1-hexanol, while others were also present in both studies but with lower VIP value [10]. This can be explained by the fact that Pranata and colleagues included pure chicken meatballs in their multivariate analysis alongside pure beef and pure wild boar meatballs, which may affect the distribution of x-variables in overall multivariate models. Aldehydes dominated the volatile profile of fried wild boar meat, with nonanal, 2(E)-decenal, hexanal, and octanal being among the most significant. Octanol was also identified, but to a lesser extent. Recently, several quality parameters for wild boar carcass were reported, including a number of volatiles [44], the most abundant of which were hexanal, 2,3-butanedione, 3-methyl-2(5H)-furanone, furan, and 1-octen-3-ol. These findings differed slightly from those of our current study, in which some of the mentioned compounds were not detected (e.g., 3-methyl-2(5H)-furanone), while others were identified but not as significant markers (e.g., furan, 1-octen-3-ol, hexanal, and 2,3-butanedione) (Table S4).

The data in Tables 3 and 4 show that volatile markers for mixture meatballs differed from those found in single meatballs. This is explained in the same way that Figure 2 is explained. It has previously been discussed that when meat from different species is mixed and heated, the volatiles produced are more diverse than those produced by single meat

Table 3. Compounds with positive coefficient values and the highest VIP value as potential volatile markers were selected from each PLS-DA class of beef meatball, rat meatballs, and their mixtures.

No	Name of volatile compound	VIP	Chemical Group
Beef meatball			
1	2-Amino-5-methyl benzoic acid	3.77596	Carboxylic acids
2	2-Amino-6-methyl benzoic acid	3.49241	Carboxylic acids
3	Heptanal	2.51042	Aldehydes
4	Benzaldehyde	1.95663	Aldehydes
5	m-Xylene, 5-ethyl	1.88318	Hydrocarbon aromatics
6	2,3-Octanedione	1.57175	Ketones
7	Ethylbenzene	1.56885	Hydrocarbon Aromatics
8	2-Undecene, 8-methyl-, (Z)-	1.34301	Alkenes
9	5-Ethyl-2-methyloctane	1.31111	Alkanes
10	Undecane, 3-methyl-	1.28084	Alkanes
Rat meatball			
1	2-Heptenal, (Z)-	4.36833	Aldehydes
2	3-Methyl-3-butenol	3.88254	Alcohols
3	Caproic acid	2.23283	Carboxylic acids
4	Pentanal	1.84006	Aldehydes
5	Cyclohexanol, 2-tert-butyl-	1.78362	Alcohols
6	Butanal, 3-methyl-	1.46912	Aldehydes
7	6-Methyl-2-heptanol	1.45298	Alcohols
8	3,6-Dimethylundecane	1.37324	Alkanes
A mixture of beef and rat meatball			
1	Nonanal	3.18424	Aldehydes
2	1-Pentanol	2.49869	Alcohols
3	Cyclobutanol	1.9398	Alcohols
4	1-Octen-3-ol	1.52296	Alcohols
5	1-Octanol	1.45283	Alcohols
6	Indole	1.40925	Heterocyclics
7	Dimethyl trisulfide	1.37674	Sulfur compounds
8	Benzene, 1,3,5-trimethyl-	1.23695	Hydrocarbon aromatics
9	2-Ethylnitrobenzene	1.17564	Hydrocarbon aromatics
10	Naphthalene	1.17032	Hydrocarbon aromatics

Table 4. Compounds with positive coefficient values and the highest VIP value as potential volatile marker selected from each PLS-DA class of beef meatball, wild boar meatballs and their mixture.

No	Name of VOC	VIP	Chemical Group
Beef Meatball			
1	2-Amino-6-methyl benzoic acid	3.46833	Carboxylic Acids
2	2-Amino-5-methyl benzoic acid	3.37975	Carboxylic Acids
3	Heptanal	2.77856	Aldeyhdes
4	m-Xylene, 5-ethyl	2.09119	Hydrocarbon Aromatics
5	Benzaldehyde	1.87289	Aldeyhdes
6	Glutaraldehyde	1.69031	Aldeyhdes
7	Ethylbenzene	1.65355	Hydrocarbon Aromatics
8	5-Ethyl-2-methyloctane	1.12383	Alkanes
9	2-Undecene, 8-methyl-, (Z)-	1.1011	Alkenes
10	5-Hepten-2-ol, 6-methyl-	0.944574	Alcohols
Wild Boar Meatball			
1	Cyclobutanol	4.35989	Alcohols
2	Undecane, 3-methyl-	2.00946	Alkanes
3	2-Methyldecane	1.85639	Alkanes
4	1-Hexanol	1.52323	Alcohols
5	Lauric acid	1.51723	Carboxylic Acid
6	Benzene, 1,2,4,5-tetramethyl-	1.32312	Hydrocarbon Aromatics
7	Diethyltoluamide	1.09429	Nitrogen Compounds
8	Benzene, 1,2,3,5-tetramethyl-	0.970724	Hydrocarbon Aromatics
9	α-Terpinolene	0.965119	Terpenoids
10	Dodecane	0.845792	Ketones
A mixture of Beef and Wild Boar Meatball			
1	Nonanal	4.15525	Aldehydes
2	1-Pentanol	2.79345	Alcohols
3	Heptanal	2.77856	Aldehydes
4	1-Octene, 3,7-dimethyl-	1.62265	Alkenes
5	Dimethyl trisulfide	1.59272	Sulfur Compounds
6	1-Heptanol	1.38125	Alcohols
7	Caproic acid	1.35531	Carboxylic Acid
8	2,4,6-Trimethyloctane	1.06567	Alkanes
9	6-Methyl-2-heptanol	0.970553	Alcohols
10	o-Cymene	0.898278	Hydrocarbon Aromatics

because different volatile precursors are mixed and subjected through the thermal process, resulting in the formation of more diverse volatiles than when meatballs are made from a single type of meat. Marker selection in each group of meatballs were done based VIP value. VIP indicates the relative importance of each variable (X variables, volatile compounds identified in this research) to the other variables (Y, meatball groups) in a PLS-DA model [25]. Since in mixture meatballs we have now more diverse volatiles (X variables) as compared to their single counterparts (Figure 2), then compounds with significant VIP value are also different. For example, nonanal was identified as significant positive marker for beef-rat mixture meatballs (Table 3), however it was negatively correlated with pure beef meatballs group and not significant for pure rat meatballs group, which makes it absent from Table 3. Almost similar case was reported by Pavlidis and co-workers (2019). In their study, methyl acetate was found to be positively correlated with the mixture of minced beef and pork. The compound was not a significant marker for the pork group and was even negatively correlated with the beef group [27].

4. Conclusions

The texture and color characteristics of meatballs measured in this study were found to be inconsistent and could not be used to identify meatballs based on raw meat compositions except at higher concentrations (50%). Volatile compound data obtained from SPME-GCMS and multivariate data analysis, on the other hand, was able to show a clear classification among meatballs made from different types of raw meat. The PLS-DA model showed that the strongest markers in beef, rat, and wild boar meatballs were 2-amino-5-methyl benzoic acid (Z)-2-heptenal, and cyclobutanol, respectively. Additionally, nonanal was consistently found as a dominant marker in meatballs made from a mixture of beef-rat and a mixture of beef wild boar. The results of this study revealed that the volatile profiles of different types of meat can be used as a basis to develop a quick analytical sensor which can detect the presence of undesired types of meat based on their volatile profiles. Further research on the verification of the volatile compounds identified as markers for each meatball group in this study is required. Verification can be accomplished by quantifying each compound using an internal standard. Because the target compounds to be quantified are already known, this future study takes a different approach than the current -omics-based research. Different analytical methods can be used, though using the same instrument (GC-MS) is the preferred method for volatiles analysis. Method validation, which includes detection limit, quantification limit, curve linearity, working range and linear range, accuracy, and recovery, must be performed prior to quantification to ensure the method fits the target compounds well.

Declarations

Author contribution statement

Lia Amalia: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Nancy Dewi Yuliana: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Purwantiningsih Sugita: Conceived and designed the experiments; Wrote the paper.

Desi Arofah: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Utami Dyah Syafitri, Anjar Windarsih, Dachriyanus, Nor Kartini Abu Bakar: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Feri Kusnandar: Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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