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Comparison of volatile flavor compounds in plant-based and real pork mince by Headspace-Gas Chromatography-Ion Mobility Spectrometry (HS-GC-IMS)

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Abstract

Improving the aroma of plant-based meat alternatives (PBMAs) is important for consumer acceptance. To explore possible strategies for mimicking the authentic meat-like aroma in plant-based pork mince (PBPM), the volatile flavor compound (VFC) profile of four PBPM samples and two real pork mince (RPM) samples were compared via headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS). Unprocessed RPM contained fewer aromatic compounds, but raw PBPM contained an abundance of VFCs. A discernible difference in the VFC profile also existed between the raw RPM samples, which were derived from common pig and free-range black pig. Similarly, a variance in the VFC profiles of different raw PBPM samples was observed. Various aromatic compounds, such as alcohols and aldehydes, are formed when RPM is cooked. Although some of these compounds can also be formed when PBPM is cooked, most VFCs present in the raw sample were reduced or eliminated during the same process. Although the impacts of steaming and stir-frying on the VFC profiles of each kind of pork differed, the similarity in the VFC profiles of PBPM and RPM increased after steaming and stir-frying.

Keywords: plant-based meat alternative; pork mince; Headspace-Gas Chromatography-Ion Mobility Spectrometry (HS-GC-IMS); volatile flavor compounds.

Practical Application: The findings in this work provide important information for further improving the aroma of PBPM through optimizing its formulation and/or the manufacturing process.

1 Introduction

There are numerous concerns regarding the production and consumption of traditional meat, including environmental, health, and animal welfare issues. As a result, plant-based meat alternatives (PBMA) have become one of the hottest topics in food-related research communities and the food industry. These products are made from plant proteins but have similar texture, nutrition, appearance, and flavor to traditional meat (Kyriakopoulou et al., 2019; He et al., 2020; Sun et al., 2021). The formation of meat-like texture relies on the structuring process of plant proteins, and it has been successfully fulfilled by the development of methods such as extrusion and shear cell technology (Dekkers et al., 2018; Sha & Xiong, 2020). With regards to the nutrition value, the new generation of PBMAs possess similar energy density, protein content, fat content, and even fatty acid profiles to corresponding traditional meat due to the ingenious design of various product formulas (Bohrer, 2019; Sun et al., 2021). Red color, similar to that of traditional fresh meat, is exhibited by the new generation of PBMAs through the use of beet juice/powder or soy leghemoglobin as coloring agents. Modern PBMAs can also change from red to a brown color, similar to cooked meat, during the cooking process (Kyriakopoulou et al., 2019; He et al., 2020). Food flavor can be divided into taste and aroma, which are related to nonvolatile and volatile flavoring agents in the food products, respectively (Guichard & Salles, 2016). Umami flavor, similar to traditional meat, can be achieved by adding appropriate condiments to the PMBAs. However, it is quite challenging to mimic the aroma of meat during PBMA manufacture and processing due to the complexity of meat aroma-related compounds (He et al., 2020; Sha & Xiong, 2020).

Thermal-induced reactions, such as the Maillard reaction, lipid oxidation and degradation, and the thermal degradation of thiamine and other compounds, are important aroma formation pathways during the cooking of meat products (Mottram, 1998; Kosowska et al., 2017). Although these reactions can be induced during the cooking process of PBMAs, slight differences between the reactions in PBMAs and traditional meat products might provoke a significant variance in the resulting aroma compounds (Kumar et al., 2017; He et al., 2020). In addition, raw meat generally only requires one heating process for consumption, while thermal and pressure treatments are mandatory during the structuring process of plant proteins in PBMA manufacturing, and further heat treatment is involved when PBMAs are cooked for human consumption (He et al., 2020). Due to the difficulties in mimicking meat aroma, the aroma of PBMAs generally lacks desirable characteristics, and this point has become the principal barrier to broad consumer acceptance (Graça et al., 2019; Sha & Xiong, 2020).

Therefore, research on the aroma and volatile flavor compounds (VFCs) of PBMA is an important and interesting scientific topic. In our previous work, the VFCs in both raw and cooked plant-based burgers were analyzed and compared to a traditional beef burger (He et al., 2021). The general

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profile of aroma-related components in these PBMA products was established, and the source of those components was also explained. Moreover, possible strategies to improve the aroma of plant-based burgers were proposed. The plant-based burger was the primary product form of PBMAs at the time of our study (Bohrer, 2019), and beef products are the highest in demand in western countries (He et al., 2020). However, more PBMA products have been developed in the past two years. Pork products are meat products with the highest in demand in China and other Asian countries (Ortega et al., 2009; Zhang et al., 2017). Plant-based pork products, especially plant-based pork mince (PBPM), are the foremost product form of PBMAs in these countries. The analysis of the VFCs in PBPM, in comparison with real pork mince (RPM), is important for the development of new strategies to mimic meat aroma in PBMAs.

Gas chromatography-mass spectrometry (GC-MS) is the widely used method for VFC analysis, and it is usually coupled with headspace-solid phase micro-extraction (HS-SPME) (Cui et al., 2020; He et al., 2021; Rahman et al., 2022). HS-SPME is a sample pre-treatment method with several merits. However, it is limited by the absorption biases of the SPME fiber (Pinho et al., 2002; Yu et al., 2008; Shi et al., 2020). Gas chromatography-ion mobility spectrometry (GC-IMS) is a technology developed in the early 1970s that has widely served as another reliable method for food VFC identification (Liu et al., 2020; Tian et al., 2020; Li et al., 2021). This method relies on GC to chromatographically separate the target compounds, which are then characterized based on gas-phase ion mobility. GC-IMS does not require time-consuming sample pretreatment, and it is usually directly coupled with headspace (HS) analysis. As a consequence, the results of HS-GC-IMS can be more accurate. The purpose of the current work, therefore, was to analyze and identify the VFCs in commercialized PBPM and compare them to traditional RPM, using HS-GC-IMS.

2 Materials and methods

2.1 Sample preparation

Two types of RPM and four types of PBPM were purchased online and transported to our lab under independent packing and frozen conditions. RPMs were derived from common pig (Pork No. 1) and free-range black pig (Pork No. 2), both consisting of half fat and half lean meat. The PBPMs were labeled as Pork No. 3, Pork No. 4, Pork No. 5, and Pork No. 6, respectively. Brief information about these samples is presented in Table 1. Three batches of pork mince were purchased biweekly and about 1.5 kg of each sample was included for every batch. Each type of pork mince was divided into three parts, the first part was kept raw (Preparation A), the second part was steamed (Preparation B), and the third part was stir-fried (Preparation C). For steaming, water was added to a steamer and heated to boiling, and the pork mince that was put on a plate in the steamer and cooked for 5 to 6 min until the internal temperature reached 74 °C. After steaming, pork mince was chilled to room temperature, and the surface moisture was absorbed with a paper towel. For stir-frying, about 10 mL of soybean oil were added to a frying pan and heated to about 220 °C. The pork mince was transferred to the pan and fried for about 5 min with occasional stirring. After frying, pork mince was chilled to room temperature, and the surface oil and moisture were absorbed with a paper towel. The same parts of each type of pork mince from the three batches were mixed and stored at −20 °C until further analysis. In total, 18 samples (six pork × three preparations/pork) were included in this work (Table 1).

2.2 Volatile flavor compound analysis

The VFCs in raw, steamed, and stir-fried samples were analyzed by a flavor analyzer (FlavourSpec®, G.A.S., Dortmund, Germany), which was based on HS-GC-IMS. Briefly, 2 g of

Table 1. Brief information of the samples used in the current work.

Sample ID[1]	Main ingredients	Nutrition value[2] (%)			ID of the tested samples		
		Total fat	Total carbohydrate	Protein	A. raw	B. steamed	C. stir-fried
Pork No. 1	Pork derived from common pig (consisting of half fat and half lean meat), salt	7.9	0.7	20.2	A-1	B-1	C-1
Pork No. 2	Pork derived from free-ranging black pig (consisting of half fat and half lean meat), salt	8.2	0.8	19.8	A-2	B-2	C-2
Pork No. 3	Water, soy protein concentrate, methyl cellulose, yeast extract, maltodextrin, potato starch, flavor, sugar, salt, soy protein isolate, mixed barley malt pulp, pea protein, rice protein, glucose, mushroom, beet red	1.3	2.2	12.5	A-3	B-3	C-3
Pork No. 4	Water, textured soybean protein, coconut oil, isolated protein powder, distarch phosphate, corn starch, yeast extract, Konjaku flour, carrageenan, mushroom powder, salt, sodium bicarbonate, sorghum red	7.9	36.2	9.1	A-4	B-4	C-4
Pork No. 5	Water, rapeseed oil, coconut oil, vinegar, wheat gluten, barley maltose powder, concentrated vegetable juice (beetroot and carrot), corn starch, salt, methyl cellulose, ascorbic acid, flavor	15.8	1.4	13.6	A-5	B-5	C-5
Pork No. 6	Water, soy protein, vegetable oil (with tocopherol), methyl cellulose, modified starch, carrageenan, flavor (with glutamic acid), caramel, beet powder, salt, yeast extract, ascorbic acid	4.1	13.1	17.7	A-6	B-6	C-6

[1]Pork No. 1 and No. 2 are real pork mince. Pork No. 3 ~ No. 6 are plant-based pork mince. [2]Data obtained from the product packaging.

thawed sample was weighed into a 20-mL HS vial, and the vial was sealed by a magnetic screw cap with polytetrafluoroethylene/silicone septa. The sealed vial was placed on the corresponding sample tray on the automatic sampler unit, and fully automated procedures were then performed. The sealed vial was incubated at 60 °C and 500 rpm conditions for 15 min, and then 500 µL HS sample was injected into the heated injector (85 °C) of the GC-IMS unit. A column (15 m in length, 0.53 mm of internal diameter, and 0.25 µm of film thickness (FS-SE-54-CB-1)) was applied for GC separation of VFCs with an isothermal model (column temperature of 60 °C). High-purity nitrogen gas (99.999%) was used as the carrier gas for GC. The flow rate was 2 mL/min during the first 2 min; then increased to 10 mL/min for 8 min; 100 mL/min for 10 min; and 150 mL/min for another 10 min (total run time for each analysis was 30 min). The separated VFCs were ionized in the IMS ionization chamber, and then IMS was analyzed with 150 mL/min high-purity nitrogen gas (99.999%) used as the drift gas. The drift tube temperature was 45 °C. Three technical replicates were included for each sample. Obtained GC-IMS data were qualitatively analyzed by VOCal processing software, based on comparing the retention index (RI) and drift time with the NIST database and the IMS database, respectively. Plug-in tools, including Reporter, Gallery plot, and Dynamic PCA, were used for corresponding analysis purposes.

3 Results and discussion

3.1 Comparison of VFCs in raw plant-based and real pork minces

The two-dimensional top view of the GC-IMS results of raw pork mince was obtained by the Reporter plug-in tool (Figure 1). Each point on either side of the reactive ion peak (RIP, the red vertical line on the left side) represents a VFC, and the color represents the concentration of the corresponding compound (white indicates lower, red indicates higher). The RPM derived from common pig (A-1) had dramatically fewer VFCs than the samples derived from free-range black pig (A-2) and PBPM (A-3 to A-6). Raw meat generally lacks the aroma properties that characterize cooked meat (Mottram, 1998); however, its VFC composition is also impacted by a series of genetic and non-genetic factors (Gąsior & Wojtycza, 2016). This might explain why more VFCs were found in sample A-2 than in A-1. In addition, the abundance of VFCs detected in raw PBPM might be related to the manufacturing process and (or) their formulas. Thermal and pressure treatments are applied during the structuring process of plant proteins, and aroma formation reactions might be induced by such treatments (He et al., 2020; He et al., 2021). In addition, food flavoring agents are generally included in the formula of PBPM (Table 1). The qualitative analysis and peak intensities of each VFC in the raw pork minces can be found in Table S1. Apart from unidentified peaks and the dimers of some compounds (formed during IMS analysis), 46 compounds were identified in these samples, including nine alcohols, 11 aldehydes, nine ketones, three acids, six esters, and eight other compounds. Only a few VFCs, including ethanol, 2-propanone, 2-butanone, 2-methylpropanal, 1-pentanol, hexanal, 2-heptanone, heptanal, and n-nonanal, existed in all six of the samples but with significantly variant peak intensities. In detail, 19 substances were identified in sample A-1, including six alcohols, six aldehydes, six ketones, and one ester, and 2-propanone, hexanal, and 2-butanone had the highest peak intensities. In contrast, 29 substances were identified in sample A-2, including eight alcohols, nine aldehydes, seven ketones, one acid, two esters, and two other substances. Acetoin, 3-methylbutanal, and hexanal were the most abundant

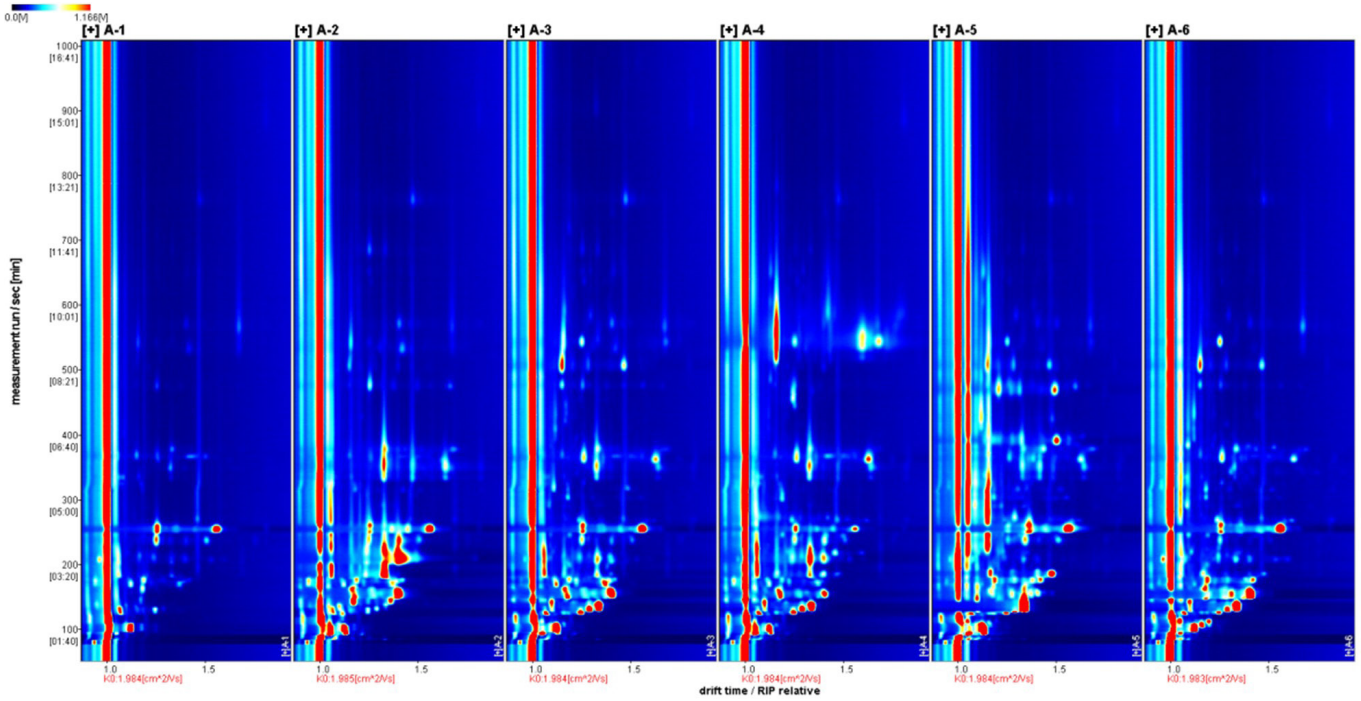


Figure 1. The two-dimensional top view of GC-IMS results of six raw pork minces.

substances. Most of the substances identified in sample A-1 or A-2 were also identified in raw pork samples by other researchers using the HS-SPME-GC-MS method (Gąsior & Wojtycza, 2016; Sun et al., 2018; Wang et al., 2020); however, substances such as ethanol, 2-propanone, 2-butanone, and 2-methylpropanal were rarely identified in raw pork samples by such method. This might be caused by the different detection abilities between the HS-SPME-GC-MS and HS-GC-IMS methods.

Twenty-nine substances, including four alcohols, nine aldehydes, eight ketones, one acid, three esters, and four other substances, were identified in sample A-3, and acetoin, 3-methylbutanal, and 2-propanone had the highest peak intensities. Twenty-seven substances, including three alcohols, eight aldehydes, seven ketones, one acid, five esters, and three other substances, were identified in sample A-4, and 1-octen-3-ol, acetoin, and ethanol were the most abundant substances. For the sample A-5, 33 substances were identified, including seven alcohols, 11 aldehydes, three ketones, three acids, three esters, and six other substances. Acetic acid, ethyl acetate, and 2-propanone were the most abundant substances. Thirty-two substances, including four alcohols, 10 aldehydes, six ketones, one acid, four esters, and seven other substances, were identified in sample A-6, and the three major substances were ethyl acetate, 3-methylbutanal, and hexanal. It should be noted that both the quantity and the main type of VFCs in these samples were notably different, which was consistent with the result of our previous comparison between the plant-based burger and beef burger (He et al., 2021).

To visualize the differences between the VFC profiles of raw plant-based and real pork mince, a gallery plot of VFC peaks selected from their GC-IMS spectra was constructed using the Gallery plot plug-in tool (Figure 2). When comparing the RPM samples derived from common pig (A-1) and free-range black pig (A-2), a series of compounds (mainly located in group 1 of Figure 2), including ethyl acetate, 3-methylbutanal, acetoin, acetic acid, (E)-2-hexen-1-ol, 1-hexanol, 2-heptenal(E), benzaldehyde, 2-pentylfuran, linalool oxide, and (E)-2-octenal, were detected in sample A-2 but not in A-1. This further illustrated the impact of genetic and non-genetic factors on the composition of VFCs in uncooked pork mince (Gąsior & Wojtycza, 2016). The other identified compounds (group 2 of Figure 2), such as 2-methyl pyrazine, 2,5-dimethyl pyrazine, 2-furfural, 2-acetylfuran, phenylacetaldehyde, methional, 2-acetylthiazole 2-methyl-1-pentanol, (E)-2-hexenal, 2-hexanone, 2-octanone, isovaleric acid, hexanoic acid, propylacetate, methyl butanoate, butyl acetate, and isoamyl acetate, were mainly detected in raw PBPMs. Among these compounds, 2-methyl pyrazine, 2,5-dimethyl pyrazine, 2-furfural, 2-acetylfuran, phenylacetaldehyde, methional, and 2-acetylthiazole are typical products of the Maillard reaction (including Strecker degradation) (Cho et al., 2010; Xu et al., 2019), and (E)-2-hexenal is a well-known lipid oxidation-derived aldehyde (Grebenteuch et al., 2021). One possible source of these products is the flavoring agents that are added to these commercialized PBPM, and another possible source is the Maillard reaction and lipid oxidation, which occur during the PBPM manufacturing process (He et al., 2021). Textured vegetable protein (TVP) is the principal ingredient of PBMAs. Dry TVP that is processed using low-moisture extrusion technology was used in the past. Currently, wet TVP, produced by high-moisture twin-screw extrusion technology, is more popular (Ryu, 2020; He et al., 2021). During wet TVP production, fats are incorporated alongside dry ingredients and water during the high-temperature and pressure extrusion process. Therefore, the Maillard reaction and lipid oxidation might be involved in this process (He et al., 2021).

Furthermore, a significant variance in VFC composition was also observed between the four PBPMs. The contents of methyl butanoate and 1-octen-3-ol were high in sample A-4 (group 2-1 of Figure 2). The contents of isovaleric acid, acetic acid, 2,5-dimethyl pyrazine, 2-methyl pyrazine, propylacetate, 2-acetylfuran, 2-acetylthiazole, 2-furfural, 2,3-butanediol, 2-methyl-1-pentanol, and cyclohexanone were high in sample A-5 (group 2-2 of Figure 2), and the contents of methional, butyl acetate, and isoamyl acetate were very high in sample A-6 (group 2-3 of Figure 2). Such variance might be caused by the differences in the formulas of these products (Table 1). For example, vinegar is one of the ingredients of Pork No. 5. Therefore, a high content of acetic acid was found in the sample A-5. In addition, mushroom powder is listed in the formula

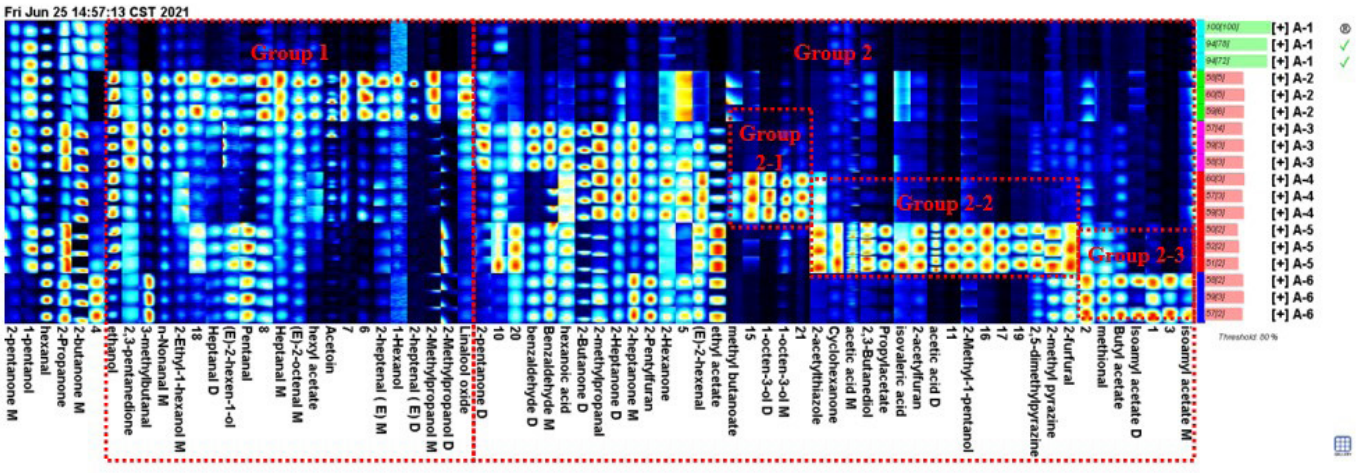


Figure 2. The gallery plot of VFC peaks selected from the GC-IMS spectra of six raw pork minces.

of Pork No. 4. Thus, a high content of 1-octen-3-ol, the major volatile metabolite of mushroom (Mau et al., 1993), was detected in the sample A-4.

Principal component analysis (PCA) was performed using the dynamic PCA plug-in tool, and the result is shown in Figure 3. It further illustrated obvious differences in the VFC profiles between raw RPM (A-1 and A-2) and raw PBPM (A-3 to A-6). However, discernible differences in the VFC profiles also existed between A-1 and A-2 and among the four raw PBPM. Samples A-3 and A-4 possessed similar VFC profiles, while sample A-5 differed the most from raw RPM.

3.2 Comparison of two cooking processes on the VFC profiles of plant-based and real pork minces

Steaming and stir-frying are universal domestic cooking methods in Chinese home kitchens (Xu et al., 2014). Therefore, plant-based and real pork minces were both steamed and stir-fried, and the impacts of these two processes on their VFC profiles were compared. The gallery plot of VFC peaks selected from the GC-IMS spectra of different sample preparations (raw, steamed, and stir-fried) of six pork minces are illustrated in Figure 4. Some VFCs were newly formed or increased after cooking, and some VFCs that existed in the raw samples were reduced. Therefore, special aroma features can be formed for these products during cooking.

A series of substances (group 1 of Figure 4a), including ethanol, 2,3-butanediol, 1-pentanol, 1-hexanol, (E)-2-hexen-1-ol, 2-ethyl-1-hexanol, 2-methylpropanol, 1-octen-3-ol, 3-methylbutanal, 2-methylpropanal, pentanal, heptanal, 2-heptenal(E), (E)-2-octenal, n-nonanal, benzaldehyde, phenylacetaldehyde, 2-propanone, 2-butanone, 2,3-pentanedione, 2-pentanone, 2-heptanone, ethyl acetate, amyl acetate, hexyl acetate, 2-pentylfuran, acetoin, butyl sulfide, and butyric acid, were newly formed or increased when RPMs derived from common pig were cooked. The contents of most of these compounds in sample B-1 were greater than in sample C-1, except the contents of ethanol, 3-methylbutanal, 2-butanone, and 2-methylpropanol in sample C-1 were higher than in sample B-1. These facts illustrate the different impacts of steaming and stir-frying on the aroma formation of RPM derived from common pig. The effects of different cooking methods, including electric oven cooking, hot-air frying, and deep-oil frying, on the aroma formation in pork loin were investigated and compared by Yang et al. (2017); however, a comprehensive investigation of the impact of steaming and stir-frying on the VFC profile of pork mince is rarely reported in the literature. Most of the compounds identified in samples B-1 and C-1 were alcohols and aldehydes, confirming they are key aroma compounds of cooked pork mince. This result is not totally consistent with the result of Yang et al. (2017), which indicated that aldehyde and pyrazine compounds are key contributors to the aroma of cooked pork loin. The different findings might be related to the difference of cooking methods used in the two studies. Most of these compounds are products of lipid oxidation and degradation (Grebenteuch et al., 2021), and this illustrates the importance of such reactions to the aroma formation of cooked pork products. However, benzaldehyde, 3-methylbutanal, and 2-pentylfuran are typical Maillard reaction products (Cho et al., 2010; Xu et al., 2019); therefore the Maillard reaction is also an essential pathway for flavor compound formation in cooked pork.

The contents of 1-hexanol, 2,3-butanediol, 2-methylpropanol, linalool oxide, cyclohexanone, 2-pentanone, and acetoin (mainly located in group 1 of Figure 4b) were high in the raw sample of pork mince derived from free-range black pig (A-2) but decreased

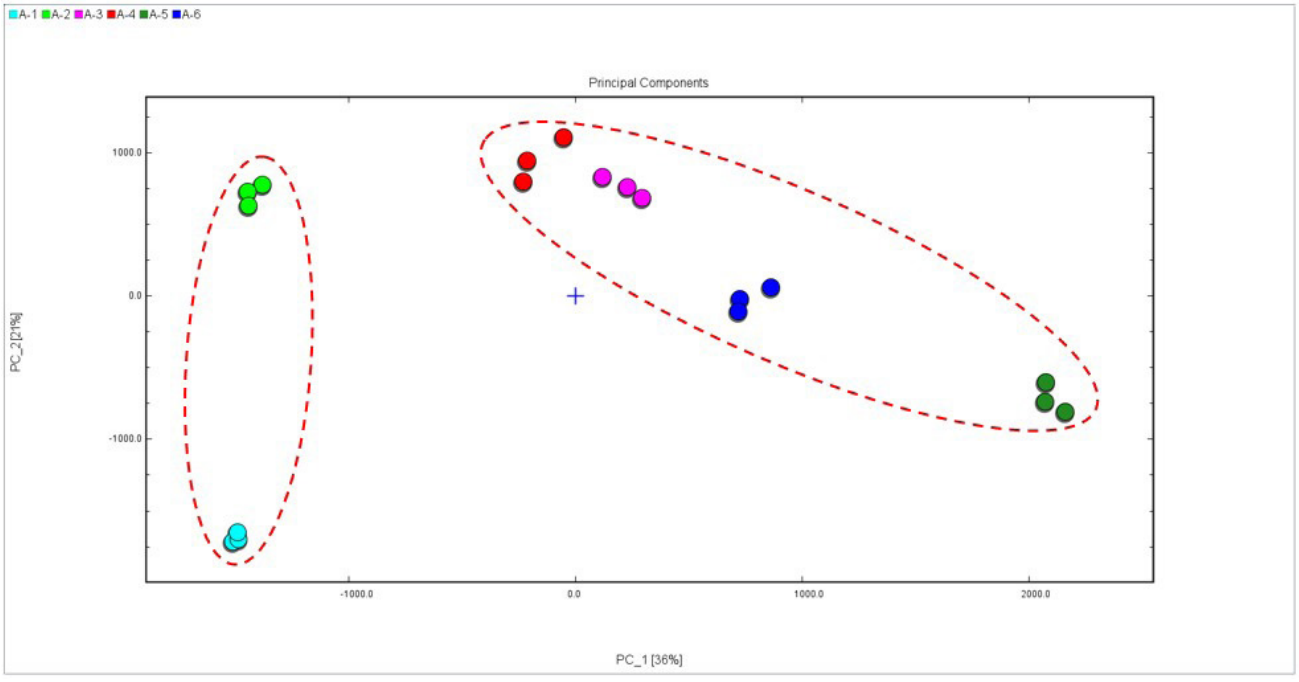


Figure 3. Principal component analysis on the VFCs profile of six raw pork minces.

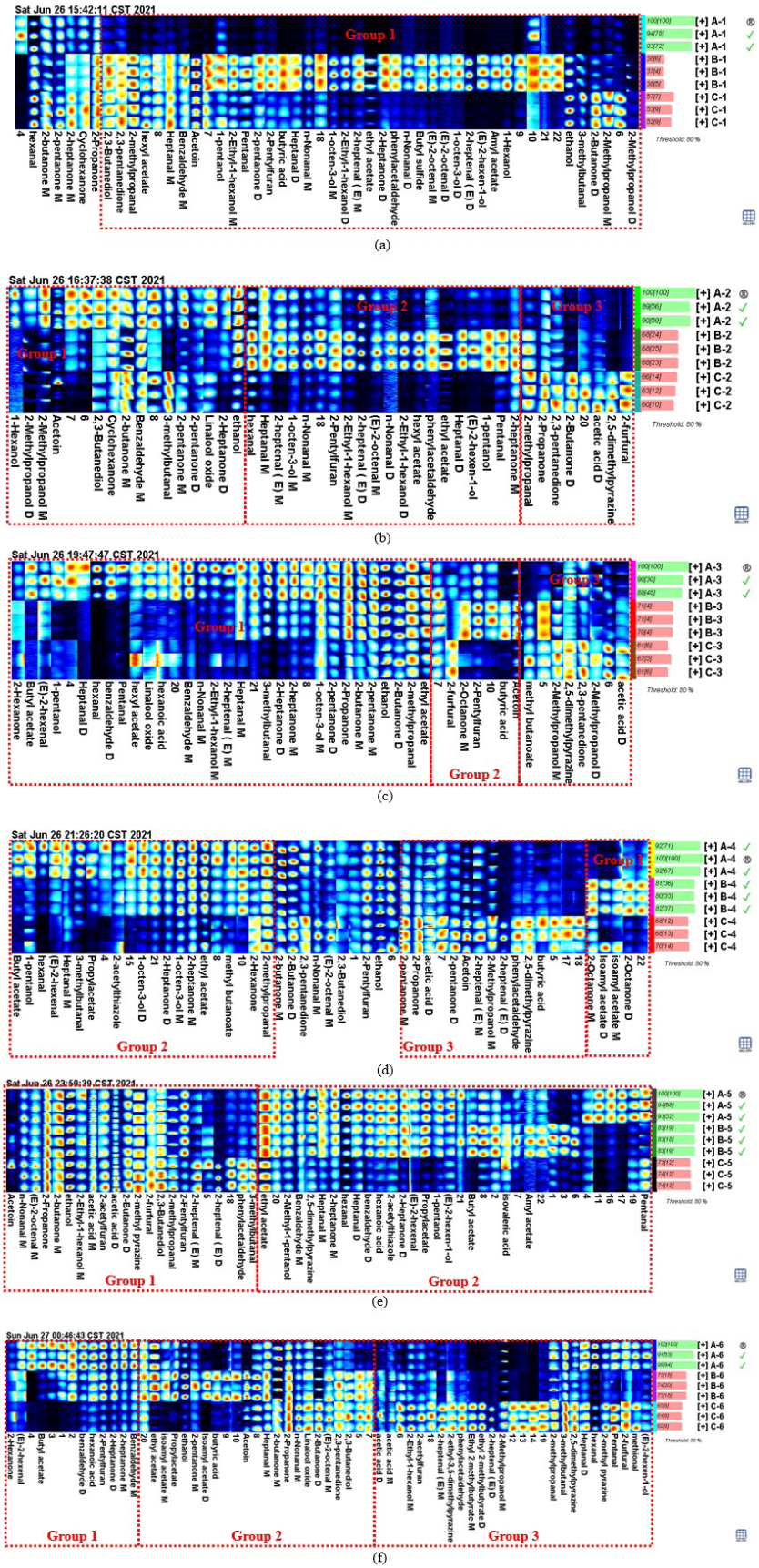


Figure 4. The gallery plot of VFC peaks selected from the GC-IMS spectra of different sample preparations (include raw (A), steamed (B), and stir-fried (C) samples of six pork minces). Figure 4a~Figure 4f represent Pork No. 1 to No. 6, respectively.

during steaming or stir-frying. The contents of (E)-2-hexen-1-ol, 1-octen-3-ol, 1-pentanol, 2-ethyl-1-hexanol, pentanal, hexanal, 2-heptenal (E), heptanal, (E)-2-octenal, n-nonanal, phenylacetaldehyde, ethyl acetate, hexyl acetate, 2-heptanone, and 2-pentylfuran (group 2 of Figure 4b) substantially increased during the steaming process of RPM derived from free-range black pig. Most of these compounds were alcohols and aldehydes and were derived from lipid oxidation and degradation (Grebenteuch et al., 2021), which is consistent with the result of steamed RPM derived from common pig. In contrast, the contents of 2-methylpropanal, 2-propanone, 2,3-pentanedione, 2-butanone, acetic acid, 2,5-dimethylpyrazine, and 2-furfural (group 3 of Figure 4b) increased during the stir-frying of authentic pork mince derived from free-range black pig. Among these compounds, 2-methylpropanal, 2,5-dimethylpyrazine, and 2-furfural are typical Maillard reaction products (Cho et al., 2010; Xu et al., 2019), illustrating the importance of such reaction in the aroma formation during stir-frying of RPM derived from free-range black pig.

Most of the compounds identified in the sample A-3, such as hexanal, heptanal, 2-methylpropanal, 3-methylbutanal, 2-heptenal (E), n-nonanal, pentanal, (E)-2-hexenal, benzaldehyde, 1-octen-3-ol, 1-pentanol, 2-ethyl-1-hexanol, ethanol, linalool oxide, 2-butanone, 2-heptanone, 2-hexanone, 2-pentanone, 2-propanone, butyl acetate, ethyl acetate, hexyl acetate, and hexanoic acid (group 1 of Figure 4c), were reduced or even disappeared after steaming or stir-frying. In contrast, the contents of 2-octanone, 2-pentylfuran, butyric acid, and acetoin (group 2 of Figure 4c) increased after steaming, and the contents of 2-methylpropanol, 2,5-dimethylpyrazine, 2,3-pentanedione, and acetic acid (group 3 of Figure 4c) increased after stir-frying. Although 2-octanone was not identified in cooked RPMs, the contents of 2-pentylfuran, butyric acid, and acetoin increased in steamed RPM derived from common pig, and the contents of 2-methylpropanol, 2,5-dimethylpyrazine, 2,3-pentanedione, and acetic acid also increased in stir-fried RPM derived from both the common pig and free-range black pig. The results indicate Pork No. 3 was partly successful in simulating the aroma formation of RPM during cooking.

During the steaming process of Pork No. 4, most of the compounds identified in the raw sample (A-4) decreased. Only 2-octanone and isoamyl acetate (group 1 of Figure 4d) were newly formed. However, both 2-octanone and isoamyl acetate were not identified in the cooked RPMs, and this illustrates the significant difference between aroma formation in steamed Pork No. 4 and RPM. The contents of 3-methylbutanal, heptanal, hexanal, 2-methylpropanal, (E)-2-hexenal, ethyl acetate, butyl acetate, methyl butanoate, propylacetate, 1-octen-3-ol, 1-pentanol, 2,3-butanediol, 2-heptanone, 2-hexanone, 2-pentylfuran, and 2-acetylthiazole (group 2 of Figure 4d) decreased, while the contents of 2,5-dimethylpyrazine, 2-heptenal(E), phenylacetaldehyde, 2-methylpropanol, 2-pentanone, 2-propanone, acetic acid, butyric acid, and acetoin (group 3 of Figure 4d) increased after stir-frying. The contents of substances that increased during stir-frying of Pork No. 4 also increased during stir-frying of RPM derived from common pig and free-range black pig. Therefore, the impact of stir-frying on the VFC profile of Pork No. 4 was more significant than the steaming process, and only stir-fried Pork No. 4 partly simulated the aroma of actual pork mince.

For Pork No. 5, only the contents of butyl acetate, isovaleric acid, and amyl acetate significantly increased after steaming, and the content of pentanal significantly decreased. Although amylacetate also increased during the steaming of RPM derived from common pig, Pork No. 5 did not simulate the aroma formation of RPM during this process. In addition, the influence of the steaming process on the aroma properties of Pork No. 5 was limited, as the VFC profiles of A-5 and B-5 were similar (similarity over 80%). For stir-fried Pork No. 5, the contents of most compounds (group 1 of Figure 4e) decreased, but the contents of 2-furfural, n-nonanal, phenylacetaldehyde, 3-methylbutanal, 2-methylpropanal, 2-heptenal (E), (E)-2-octenal, 2-acetylfuran, 2,3-butanediol, 2-butanone, 2-methyl pyrazine, acetoin, and acetic acid (group 2 of Figure 4e) increased. Interestingly, 2-acetylfuran and 2-methyl pyrazine were not identified in cooked RPMs, but the other substances also increased in stir-fried RPM derived from the common pig and free-range black pig. This indicates Pork No. 5 can partly simulate the aroma formation of authentic pork mince during the stir-frying process.

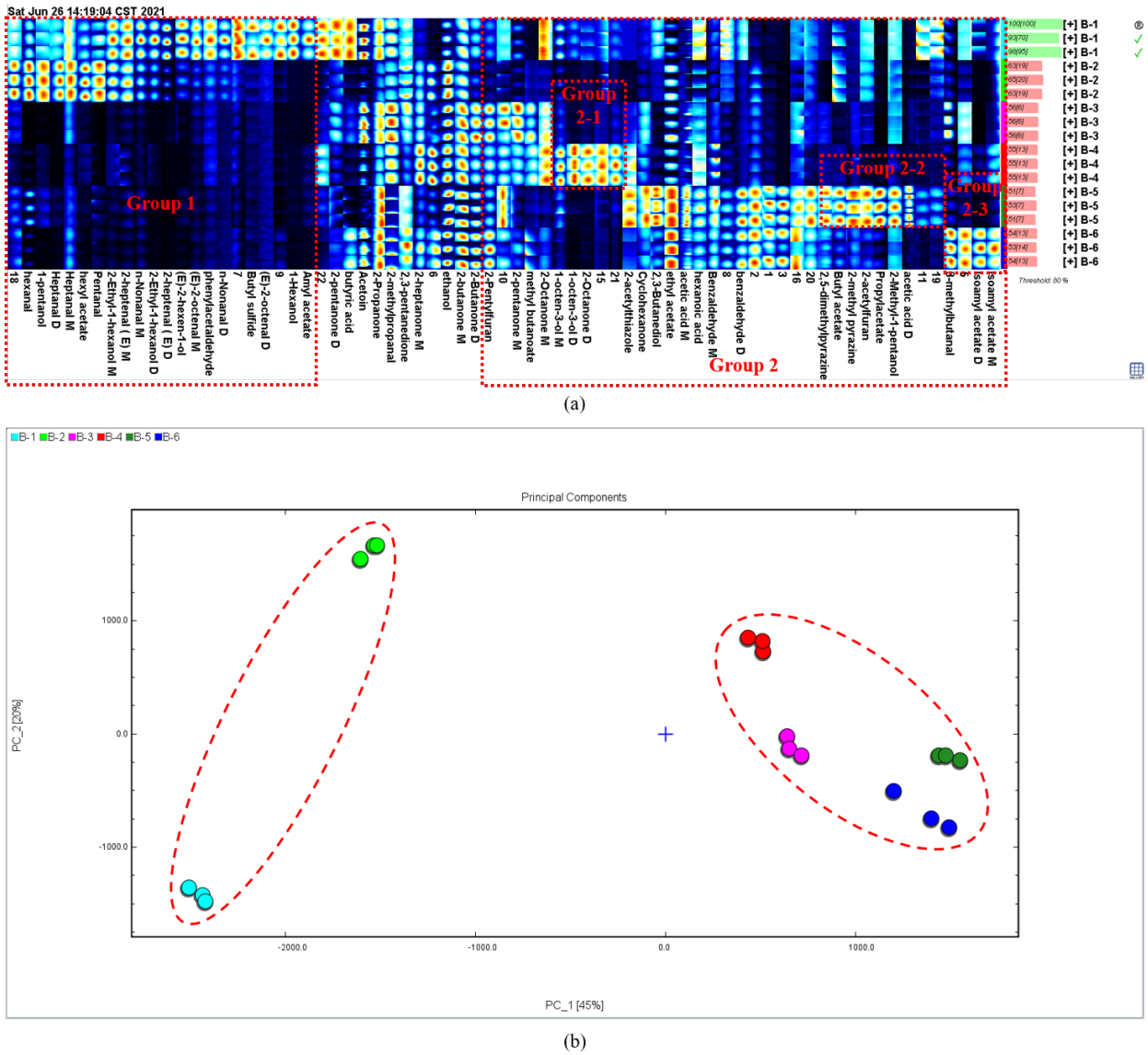
The contents of 2-hexanone, 2-heptanone, hexanoic acid, (E)-2-hexenal, butyl acetate, benzaldehyde, and 2-pentylfuran (group 1 of Figure 4f) decreased during the cooking of Pork No. 6, especially stir-frying. Steamed Pork No. 6 exhibited an increase in acetoin, ethyl acetate, propylacetate, isoamyl acetate, butyric acid, ethanol, 2-pentanone, 2-propanone, 2,3-pentanedione, and 2,3-butanediol (group 2 of Figure 4f), while stir-fried Pork No. 6 exhibited an increase in 2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2-methyl pyrazine, 2-furfural, 2-heptenal (E), (E)-2-octenal, 3-methylbutanal, n-nonanal, pentanal, phenylacetaldehyde, 2-propanone, 2-butanone, 2,3-pentanedione, 2-ethyl-1-hexanol, 2-methylpropanol, 2,3-butanediol, linalool oxide, 2-acetylfuran, acetic acid, and ethyl 2-methylbutyrate (group 3 of Figure 4f). The impact of the cooking process on the VFC profile of Pork No. 6 was more obvious, as the contents of more substances increased. In addition, most of these increased substances were also identified in cooked RPM, which suggests Pork No. 6 can partly simulate the aroma formation of authentic pork mince during steaming and stir-frying.

To summarize, an obvious difference in the VFC profiles of steamed and stir-fried pork mince samples was found. Abundant aroma substances were generated when RPM samples were cooked, especially steamed RPM derived from the common pig (B-1). In contrast, the impact of the cooking process on the VFC profiles of PBPM was not so obvious, especially in steamed Pork No. 4 and Pork No. 5, as indicated by the similarities (over 80%) between B-4 and A-4, as well as B-5 and A-5 (Figure 4d-4e). Among the substances the newly formed or increased substances during the cooking of PBPMs, most are products of the Maillard reaction (Cho et al., 2010; Xu et al., 2019) or lipid oxidation (Grebenteuch et al., 2021). Therefore, these reactions are also important routes of aroma formation during the cooking of PBPMs. However, the products of such reactions in different pork mince samples varied significantly.

3.3 Comparison of VFCs in cooked plant-based and real pork minces

The qualitative results and peak intensities of each VFC in the steamed plant-based and real pork minces are presented in Table S2. In total, 48 compounds were identified in these samples, including nine alcohols, 11 aldehydes, nine ketones, four acids, seven esters, and eight other compounds. Ethanol, 2-methylpropanal, 3-methylbutanal, heptanal, n-nonanal, 2-butanone, 2-heptanone, 2-propanone, ethyl acetate, and 2-pentylfuran existed in all six samples. There were 31 substances, including eight alcohols, 10 aldehydes, seven ketones, one acid, three esters, and two others, identified in sample B-1, of which acetoin, 2-propanone, and 1-octen-3-ol had the highest peak intensities. However, only 27 substances, including six alcohols, 10 aldehydes, seven ketones, one acid, two esters, and one other compound, were identified in sample B-2, and the three major VFCs were hexanal, acetoin, and ethyl acetate. In total, 23, 28, 34, and 31 VFCs were identified in the steamed PBPM samples B-3, B-4, B-5, and B-6, respectively. The three most abundant substances in sample B-3 were acetoin, 2-propanone, and ethyl acetate. In sample B-4, they were acetoin, 1-octen-3-ol, and ethyl acetate. The three most abundant compounds in sample B-5 were acetic acid, ethyl acetate, and 2-propanone, and in sample B-6, they were acetic acid, acetoin, and ethyl acetate. Therefore, the VFC profiles of the steamed plant-based and real pork minces were significantly different.

The gallery plot of VFC peaks selected from the GC-IMS spectra of steamed plant-based and real pork mince is presented in Figure 5a. The plot shows substances, such as hexanal, heptanal, 2-heptenal(E), (E)-2-octenal, n-nonanal, pentanal, phenylacetaldehyde, (E)-2-hexen-1-ol, 1-hexanol, 1-pentanol, 2-ethyl-1-hexanol, amyl acetate, hexyl acetate, and butyl sulfide (group 1 of Figure 5a), mainly existed in the steamed real pork samples. Among these compounds, the contents of pentanal,



(a)

(b)

Figure 5. The gallery plot of VFC peaks selected from the GC-IMS spectra (a) and principal component analysis on the VFCs profile (b) of six steamed pork minces.

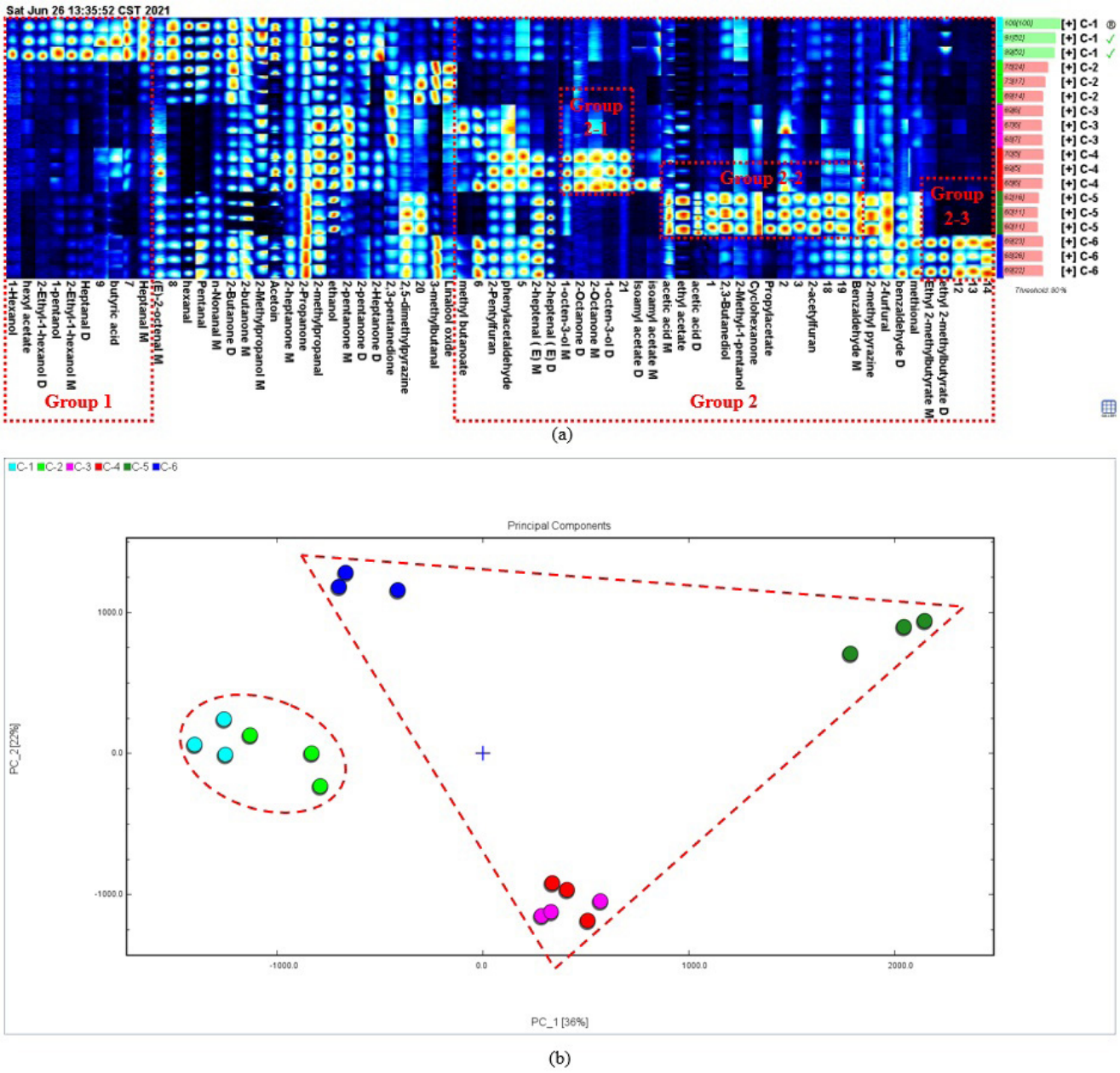


Figure 6. The gallery plot of VFC peaks selected from the GC-IMS spectra (a) and principal component analysis on the VFCs profile (b) of six stir-fried pork minces.

hexanal, heptanal, 1-pentanol, and hexyl acetate in the steamed RPM derived from free-range black pig (B-2) were higher than in the sample derived from common pig (B-1), while the contents of (E)-2-octenal, 2-heptenal(E), n-nonanal, phenylacetaldehyde, 1-hexanol, 2-ethyl-1-hexanol, (E)-2-hexen-1-ol, amyl acetate, and butyl sulfide in sample B-1 were higher than those in sample B-2. Other compounds, including 2,5-dimethylpyrazine, 2-methyl pyrazine, acetic acid, hexanoic acid, ethyl acetate, butyl acetate, isoamyl acetate, methyl butanoate, propylacetate, 2-pentylfuran, 2-acetylfuran, 2-acetylthiazole, 3-methylbutanal, benzaldehyde, 2-octanone, cyclohexanone, 2-methyl-1-pentanol, 1-octen-3-ol, and 2,3-butanediol (group 2 of Figure 5a), were mainly identified in the steamed plant-based pork samples. Among these substances, 1-octen-3-ol and 2-octanone (group 2-1 of Figure 5a) were characteristic of B-4, as their contents were significantly higher than in other plant-based pork samples. Compounds, such as 2,5-dimethylpyrazine, 2-methyl pyrazine, butyl acetate, propylacetate, 2-acetylfuran, 2-methyl-1-pentanol, acetic acid, and 2,3-butanediol (group 2-2 of Figure 5a), mainly existed in sample B-5 and with very high contents. Compounds 3-methylbutanal and isoamyl acetate (group 2–3 of Figure 5a) were distinguishing components of sample B-6. These results further highlight the differences between the VFC profiles of the four kinds of steamed PBPMs. However, the PCA results (Figure 5b) show these differences are slight, compared to the raw samples. The PCA results also illustrate the VFC profiles of steamed PBPMs were significantly different from steamed RPMs.

Table S3 presents the qualitative analysis and peak intensities of each VFC in the stir-fried samples. Similar to the steamed samples, 48 compounds were identified in these samples, including nine alcohols, 11 aldehydes, eight ketones, four acids, seven esters, and nine other compounds. Here, ethanol, 2-methylpropanal, 3-methylbutanal, hexanal, n-nonanal, 2-butanone, 2-heptanone, 2-propanone, acetoin, and ethyl acetate were present in all six stir-fried samples. Interestingly, the amount of identified VFCs and the amount of co-existing VFCs in stir-fried samples were similar to steamed samples. However, based on the description in section 2.2, the impact of stir-frying on the aroma formation of these pork mince samples was not identical to the steaming process. When comparing the VFC profiles of different stir-fried samples, the number of identified substances in samples C-1 through C-6 was 29, 26, 19, 25, 33, and 33, respectively, and their compositions varied. The three most abundant substances were acetoin, 2-propanone, and hexanal in sample C-1; 3-methylbutanal, acetoin, and 2-propanone in sample C-2; acetoin, 2-propanone, and ethyl acetate in sample C-3; acetoin, 1-octen-3-ol, and 2-propanone in sample C-4; acetic acid, ethyl acetate, and 2-propanone in sample C-5; and acetic acid, acetoin, and 3-methylbutanal in sample C-6. These results are different from the results of steamed samples, which confirms the different impacts of steaming and stir-frying on the aroma formation of plant-based and real pork minces. In addition, the amount of identified VFCs in cooked Pork No. 3 was less than other cooked samples, regardless of the cooking technique. This might be because Pork No. 3 has less fat content (Table 1).

Figure 6a displays the gallery plot of VFC peaks selected from the GC-IMS spectra of stir-fried plant-based and real pork mince samples. Substances, such as 1-hexanol, 2-ethyl-1-hexanol, 1-pentanol, heptanal, hexyl acetate, and butyric acid (group 1 of Figure 6a), were mainly identified in stir-fried pork mince derived from the common pig (C-1). A large number of esters, including ethyl acetate, isoamyl acetate, methyl butanoate, propylacetate, and ethyl 2-methylbutyrate, were mainly identified in stir-fried PBPMs. In addition, substances such as 1-octen-3-ol, 2-methyl-1-pentanol, 2,3-butanediol, 2-acetylfuran, 2-pentylfuran, 2-heptenal(E), benzaldehyde, methional, 2-furfural, 2-octanone, cyclohexanone, 2-methyl pyrazine, and acetic acid (group 2 of Figure 6a), were predominantly found in stir-fried PBPMs. Among these compounds, 1-octen-3-ol and 2-octanone (group 2-1 of Figure 6a), were the characteristic compositions of sample C-4, with higher contents than other samples. Acetic acid, ethyl acetate, 2,3-butanediol, 2-methyl-1-pentanol, propylacetate, and 2-acetylfuran (group 2-2 of Figure 6a) were the characteristic compounds of sample C-5, and methional and ethyl 2-methylbutyrate (group 2-3 of Figure 6a) were characteristic of sample C-6. The PCA results (Figure 6b) indicate the similarity between the VFC profiles of stir-fried RPMs, derived from the common pig and free-range black pig, was greater than between raw and corresponding steamed samples. However, the similarity between the VFC profiles of stir-fried PBPMs was less than that between steamed samples. The exception was the similarity between samples C-3 and C-4, which was higher than between samples B-3 and B-4. In addition, the overall similarity between the VFC profiles of stir-fried plant-based and real pork minces was greater than that between raw and steamed samples.

PCA results of the VFC profiles of all 18 samples are presented in Figure 7. The difference in the VFC profiles between the raw, steamed, and stir-fried Pork No. 6 was more obvious than other plan-based pork mince samples. The similarity between the VFC profiles of steamed and raw samples of the four kinds

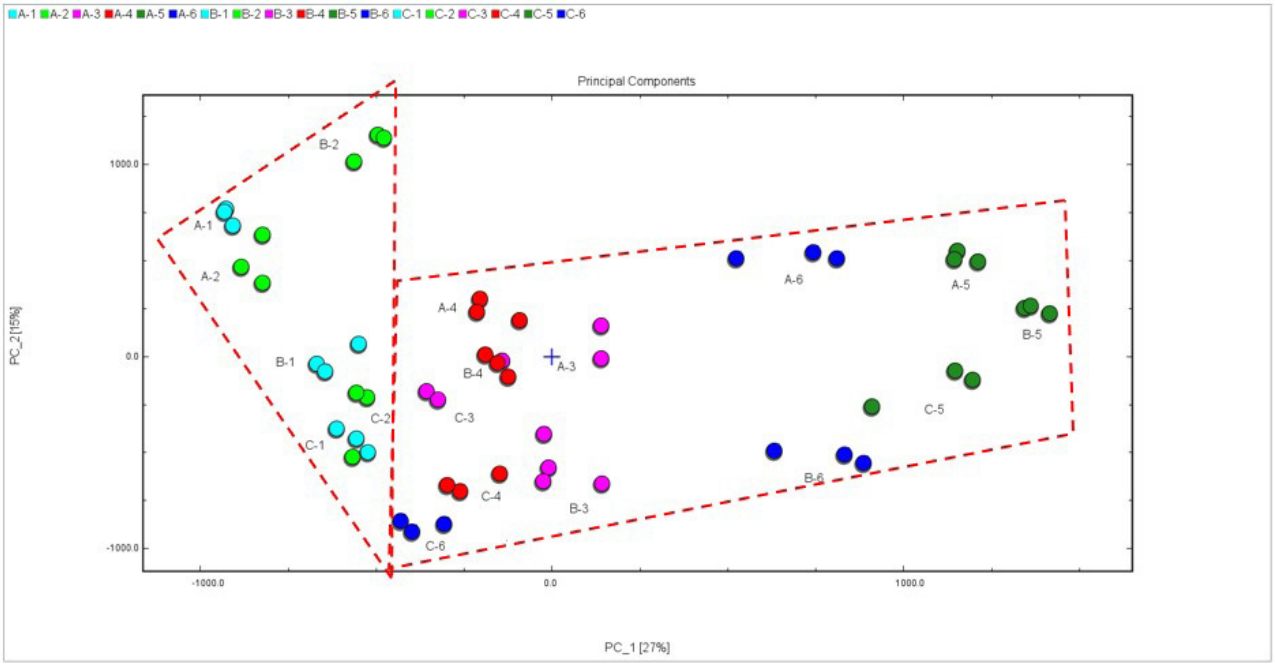


Figure 7. Principal component analysis on the VFCs profile of six pork minces under raw (A), steamed (B), and stir-fried (C) preparations.

of PBPMs was greater than the similarity between stir-fried and raw samples. The VFC profiles of steamed and stir-fried RPM derived from the common pig were similar and easily distinguished from the raw sample. The difference in the VFC profiles of real and PBPMs was most obvious for raw samples, and it became inconspicuous after stir-frying. The exception was stir-fried Pork No. 5, which was clearly distinguished from other stir-fried samples.

4 Conclusion

Raw RPM generally contains few aroma compounds; however, raw PBPM contained more VFCs, which were attributed to either the addition of flavoring agents or aroma formation reactions during manufacturing. Therefore, a discernible difference in the VFC profiles existed between raw RPM and unprocessed PBPM. The VFC profile of pork products is determined by a series of genetic and non-genetic factors; therefore, there was a discernible difference between raw RPM derived from the common pig and free-range black pig. Similarly, a variance in the VFC profiles of different raw PBPMs was due to the discrepancies in their formulas and manufacturing processes. The impact of the cooking process on the VFC profiles of RPM and PBPM was different. A number of important aroma compounds are formed through the Maillard reaction and lipid oxidation during cooking, and alcohols and aldehydes are key aroma compounds of cooked authentic pork mince. Although the cooking of PBPM can also induce the formation of some aroma compounds, similar to RPM, the contents of some VFCs in the raw sample diminished or disappeared. The impact of steaming and stir-frying on the VFC profiles of each kind of pork mince was also different; however, the similarity between PBPM and RPM increased after steaming and stir-frying, and each type of PBPM partly simulated the aroma formation of authentic pork mince during cooking. Optimization of the formula and the manufacturing process of PBPM might be effective strategies to further improve its aroma similarity to authentic pork mince, and this will be investigated in our future studies.

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References

Bohrer, B. M. (2019). An investigation of the formulation and nutritional composition of modern meat analogue products. *Food Science and Human Wellness*, 8(4), 320-329. http://dx.doi.org/10.1016/j.fshw.2019.11.006.

Cho, I. H., Lee, S., Jun, H. R., Roh, H. J., & Kim, Y. S. (2010). Comparison of volatile Maillard reaction products from tagatose and other reducing sugars with amino acids. *Food Science and Biotechnology*, 19(2), 431-438. http://dx.doi.org/10.1007/s10068-010-0061-7.

Cui, L., Xu, J., Feng, Z., Yan, M., Piao, X., Yu, Y., Hou, W., Jin, Y., & Wang, Y.-P. (2020). Simultaneous determination and difference evaluation of volatile components generated from ginseng fruit by HS-SPME coupled with GC-MS according to fruit color. *Food Science and Technology*, 40(3), 532-536. http://dx.doi.org/10.1590/fst.26718.

Dekkers, B. L., Boom, R. M., & van der Goot, A. J. (2018). Structuring processes for meat analogues. *Trends in Food Science & Technology*, 81, 25-36. http://dx.doi.org/10.1016/j.tifs.2018.08.011.

Gąsior, R., & Wojtycza, K. (2016). Sense of smell and volatile aroma compounds and their role in the evaluation of the quality of products of animal origin-a review. *Annals of Animal Science*, 16(1), 3-31. http://dx.doi.org/10.1515/aoas-2015-0047.

Graça, J., Godinho, C. A., & Truninger, M. (2019). Reducing meat consumption and following plant-based diets: current evidence and future directions to inform integrated transitions. *Trends in Food Science & Technology*, 91, 380-390. http://dx.doi.org/10.1016/j.tifs.2019.07.046.

Grebenteuch, S., Kroh, L. W., Drusch, S., & Rohn, S. (2021). Formation of secondary and tertiary volatile compounds resulting from the lipid oxidation of rapeseed oil. *Foods*, 10(10), 2417. http://dx.doi.org/10.3390/foods10102417. PMid:34681465.

Guichard, E., & Salles, C. (2016). Retention and release of taste and aroma compounds from the food matrix during mastication and ingestion. In P. Etiévant, E. Guichard, C. Salles & A. Voilley (Eds.), *Flavor: from food to behaviors, wellbeing and health* (pp. 3-22). Amsterdam: Elsevier. http://dx.doi.org/10.1016/B978-0-08-100295-7.00001-3.

He, J., Evans, N. M., Liu, H., & Shao, S. (2020). A review of research on plant-based meat alternatives: driving forces, history, manufacturing, and consumer attitudes. *Comprehensive Reviews in Food Science and Food Safety*, 19(5), 2639-2656. http://dx.doi.org/10.1111/1541-4337.12610. PMid:33336979.

He, J., Liu, H., Balamurugan, S., & Shao, S. (2021). Fatty acids and volatile flavor compounds in commercial plant-based burgers. *Journal of Food Science*, 86(2), 293-305. http://dx.doi.org/10.1111/1750-3841.15594. PMid:33472280.

Kosowska, M., Majcher, M. A., & Fortuna, T. (2017). Volatile compounds in meat and meat products. *Food Science and Technology*, 37(1), 1-7. http://dx.doi.org/10.1590/1678-457x.08416.

Kumar, P., Chatli, M., Mehta, N., Singh, P., Malav, O., & Verma, A. K. (2017). Meat analogues: health promising sustainable meat substitutes. *Critical Reviews in Food Science and Nutrition*, 57(5), 923-932. http://dx.doi.org/10.1080/10408398.2014.939739. PMid:25898027.

Kyriakopoulou, K., Dekkers, B., & van der Goot, A. J. (2019). Plant based meat analogues. In C. M. Galanakis (Ed.), *Sustainable meat production and processing* (pp. 103-126). London: Elsevier. http://dx.doi.org/10.1016/B978-0-12-814874-7.00006-7.

Li, M., Du, H., & Lin, S. (2021). Flavor changes of tricholoma matsutake singer under different processing conditions by using HS-GC-IMS. *Foods*, 10(3), 531. http://dx.doi.org/10.3390/foods10030531. PMid:33806370.

Liu, D., Bai, L., Feng, X., Chen, Y. P., Zhang, D., Yao, W., Zhang, H., Chen, G., & Liu, Y. (2020). Characterization of Jinhua ham aroma profiles in specific to aging time by Gas Chromatography-Ion Mobility Spectrometry (GC-IMS). *Meat Science*, 168, 108178. http://dx.doi.org/10.1016/j.meatsci.2020.108178. PMid:32417671.

Mau, J. L., Beelman, R. B., & Ziegler, G. R. (1993). Factors affecting 1-octen-3-ol in mushrooms at harvest and during postharvest storage. *Journal of Food Science*, 58(2), 331-334. http://dx.doi.org/10.1111/j.1365-2621.1993.tb04268.x.

Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry*, 62(4), 415-424. http://dx.doi.org/10.1016/S0308-8146(98)00076-4.

Ortega, D. L., Wang, H. H., & Eales, J. S. (2009). Meat demand in China. *China Agricultural Economic Review*, 1(4), 410-419. http://dx.doi.org/10.1108/17561370910989248.

Pinho, O., Ferreira, I. M., & Ferreira, M. A. (2002). Solid-phase microextraction in combination with GC/MS for quantification of the major volatile free fatty acids in ewe cheese. *Analytical Chemistry*, 74(20), 5199-5204. http://dx.doi.org/10.1021/ac020296m. PMid:12403571.

Rahman, F. U., Nawaz, M. A., Liu, R., Sun, L., Jiang, J., Fan, X., Liu, C., & Zhang, Y. (2022). Evaluation of volatile aroma compounds from Chinese wild grape berries by headspace-SPME with GC-MS. *Food Science and Technology*, 42, e54320. http://dx.doi.org/10.1590/fst.54320.

Ryu, G.-H. (2020). Extrusion cooking of high-moisture meat analogues. In G. M. Ganjyal (Ed.), *Extrusion cooking: cereal grains processing* (2nd ed., pp. 205-224). Oxford: Elsevier/Cereals & Grains Association. http://dx.doi.org/10.1016/B978-0-12-815360-4.00007-9.

Sha, L., & Xiong, Y. L. (2020). Plant protein-based alternatives of reconstructed meat: science, technology, and challenges. *Trends in Food Science & Technology*, 102, 51-61. http://dx.doi.org/10.1016/j.tifs.2020.05.022.

Shi, Y., Chen, L., Huang, J., Ma, Z., Hu, Q., Zhu, F., Zheng, J., & Ouyang, G. (2020). The effect of different binders on the comprehensive performance of solid phase microextraction fiber. *Analytica Chimica Acta*, 1140(12), 50-59. http://dx.doi.org/10.1016/j.aca.2020.09.048. PMid:33218489.

Sun, C., Ge, J., He, J., Gan, R., & Fang, Y. (2021). Processing, quality, safety, and acceptance of meat analogue products. *Engineering*, 7(5), 674-678. http://dx.doi.org/10.1016/j.eng.2020.10.011.

Sun, Y., Fu, M., Li, Z., & Peng, X. (2018). Evaluation of freshness in determination of volatile organic compounds released from pork by HS-SPME-GC-MS. *Food Analytical Methods*, 11(5), 1321-1329. http://dx.doi.org/10.1007/s12161-017-1109-6.

Tian, X., Li, Z. J., Chao, Y. Z., Wu, Z. Q., Zhou, M. X., Xiao, S. T., Zeng, J., & Zhe, J. (2020). Evaluation by electronic tongue and headspace-GC-IMS analyses of the flavor compounds in dry-cured pork with different salt content. *Food Research International*, 137, 109456. http://dx.doi.org/10.1016/j.foodres.2020.109456. PMid:33233132.

Wang, Z., Cai, R., Yang, X., Gao, Z., & Yue, T. (2020). Changes in aroma components and potential Maillard reaction products during the stir-frying of pork slices. *Food Control*, 123(1), 107855.

Xu, F., Zheng, Y., Yang, Z., Cao, S., Shao, X., & Wang, H. (2014). Domestic cooking methods affect the nutritional quality of red cabbage. *Food Chemistry*, 161, 162-167. http://dx.doi.org/10.1016/j.foodchem.2014.04.025. PMid:24837935.

Xu, X., Zheng, Y., Song, H., Gong, L., & Pan, W. (2019). The effects of enzymatic hydrolysis degree of bovine bone marrow extract on flavor generation via the Maillard reaction. *Journal of Food Measurement and Characterization*, 13(1), 521-535. http://dx.doi.org/10.1007/s11694-018-9966-2.

Yang, Z., Lu, R., Song, H., Zhang, Y., Tang, J., & Zhou, N. (2017). Effect of different cooking methods on the formation of aroma components and heterocyclic amines in pork loin. *Journal of Food Processing and Preservation*, 41(3), e12981. http://dx.doi.org/10.1111/jfpp.12981.

Yu, A. N., Sun, B. G., Tian, D. T., & Qu, W. Y. (2008). Analysis of volatile compounds in traditional smoke-cured bacon (CSCB) with different fiber coatings using SPME. *Food Chemistry*, 110(1), 233-238. http://dx.doi.org/10.1016/j.foodchem.2008.01.040. PMid:26050188.

Zhang, W., Naveena, B. M., Jo, C., Sakata, R., Zhou, G., Banerjee, R., & Nishiumi, T. (2017). Technological demands of meat processing-an Asian perspective. *Meat Science*, 132, 35-44. http://dx.doi.org/10.1016/j.meatsci.2017.05.008. PMid:28648604.

Supplementary Material

Supplementary material accompanies this paper.

Table S1. The peak intensity of VFCs that identified in six raw pork minces by GC-IMS.

Table S2. The peak intensity of VFCs that identified in six steamed pork minces by GC-IMS.

Table S3. The peak intensity of VFCs that identified in six stir-fried pork minces by GC-IMS.

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