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Title: Characterization and differentiation of boiled pork from Tibetan, Sanmenxia and Duroc × (Landrac × Yorkshire) pigs by volatiles profiling and chemometrics analysis

Article Type: Research Articles

Keywords: GC-MS/O; E-nose; pork breeds; odour-active compounds; potential flavour markers

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Abstract: To characterize and differentiate boiled pork from three different breeds of pig (Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire)), the volatile compounds in each were analysed by gas chromatography-olfactometry-mass spectrometry (GC-MS/O) and electronic nose (E-nose) combined with chemometrics analysis. In total, 61 volatile compounds were identified, among which 25 compounds were selected as odour-active compounds in boiled pork. Moreover, seven odour-active compounds (hexanal, nonanal, 1-octen-3-ol, dimethyl disulphide, heptanal, 2-pentylfuran and 2-ethylfuran) were the main contributors to the integral flavour of boiled pork due to their higher odour activity values (OAVs) ranging from 17.3-524.2. The odour-active compounds were examined by principal component analysis (PCA), agglomerative hierarchical clustering (AHC) and partial least squares-discriminant analysis (PLS-DA). The results showed that boiled pork from the three pig breeds could be clearly distinguished, and twelve odour-active compounds, including (E,E)-2,4-decadienal, ethyl hexanoate, dimethyl disulphide, hexanal, 2-acetylthiazole, (E)-2-nonenal, 1-octen-3-ol, (E,E)-2,4-nonadienal, heptanal, (E)-2-octen-1-ol, styrene and (E)-2-octenal, were determined as potential flavour markers for discrimination. This study indicated that GC-MS/O and E-nose with chemometrics analysis are feasible methods to characterize and discriminate boiled pork from three pig breeds.

Aug. 19th 2019

Dr. A. Sant'Ana, Editor-in-Chief

Food Research International

Dear Dr. A. Sant'Ana,

Please find submitted our research article entitled “Characterization and differentiation of boiled pork from different breeds of pigs by volatiles profiling and chemometrics analysis” by Dong Han, Chunhui Zhang, Marie-Laure Fauconnier and Si Mi for publication in *Food Research International*.

The aim of this study was to characterize the volatiles profile of boiled pork from Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire) pigs by application of GC-MS/O, followed by confirmation of the potential flavour compounds by chemometrics analysis. Moreover, multivariate statistical methods were used to explore the feasibility of differentiating boiled pork from Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire) pigs using volatile compounds. The results of this study will provide a better understanding of the odour-active compounds in boiled pork from different breeds of pigs and can be expected to establish a new method to discriminate pork from different pig breeds. This article contains original data that is not being considered for publication, in whole or in part, elsewhere. All of the authors have reviewed and approved the manuscript and there are no conflicts of interest to report. We look forward to the review and sincerely hope that our paper will be of sufficient merit to be published in *Food Research International*.

Thank you in advance for your consideration.

Sincerely,

Chun-Hui Zhang, PhD

Response to Editor's Comments

Editor's comments:

Point: The list of references must be updated. There was a lot done in this field in the last 3 years (2017-2019). Making reference to recent work in the field is particularly key to highlight the current context of the present manuscript and to make it more comprehensive and to highlight the novelty to the readers as well as its assessment and contribution to the field. Please, address this request by adding new data, critical analysis and not by simply citing papers published in this subject in the mentioned years.

Response: Many thanks for your valuable comments. According to your suggestion, the introduction has been completely revised and as follows (**Line 44-137**). Fonts marked in red are added, updated and modified sections. Additionally, the paper has been edited by **Elsevier Language Editing Services** to read smoothly.

1. Introduction

According to a United States Department of Agriculture (USDA) report, global pork production is expected to rise to 113.0 million tons. China has the largest pork share in the global market, accounting for 48.7% (55.0 million tons) of total production in the whole world. Moreover, pork is popular with consumers due to its sensory attributes, such as tender texture, rich nutritional composition (Purriños, Franco, Carballo, & Lorenzo, 2012; Sivakumar 2016) and unique flavour (Straadt, Aaslyng, & Bertram, 2013). Flavour is one of the most important sensory attributes for consumers **to judge the quality of** pork (Wang, Song, Zhang, Tang, & Yu, 2016) **and mainly associated with the generation of volatile compounds (Zhao et al., 2017).** **Previous studies have indicated that** over 1000 volatile compounds have been identified in meat and meat products, including aldehydes, ketones, alcohols, acids, esters, hydrocarbons, ethers, heterocyclic compounds and sulphur compounds (Shahidi 1998). **These compounds are mainly derived from a complex series of chemical reactions (e.g., lipid oxidation, the Maillard reaction and lipid-Maillard interactions) between precursors, intermediate reaction products and degradation**

products (Jayasena, Ahn, Nam, & Jo, 2013).

To explore the composition, origin and formation of volatile compounds in different pork products, many studies have been performed in recent years. A total of 149 volatile compounds (25 aldehydes, 18 phenols, 12 alcohols, 16 terpenes, 27 aromatic hydrocarbons, 18 aliphatic hydrocarbons, 17 ketones, 9 esters and 7 acids) were identified from dry-cured hams using four different processing methods, among which aldehydes and phenols were the more abundant volatiles (Petričević, Radovčić, Lukić, Listeš, & Medić, 2018). The volatile compounds in six dry-cured meat products were detected using a GC/MS technique; these compounds were then used to identify the possible source of the typical volatiles (Domínguez et al., 2019). Due to lipid oxidation, brine permeation and carbohydrate fermentation, the levels of volatile compounds under high pressure treatment contributed were more than 70% of the typical aroma, except for acetic acid (Yang, Sun, Pan, Wang, & Cao, 2018). Although a large number of volatile compounds associated with different processing technologies for specific types of pork have been fully analysed, information on volatile profiles in different varieties of processed pork products are still lacking.

As reported, the intramuscular fat, colour and flavour of pork from different pig breeds have been studied by many researchers (Lee et al. 2012; Lu, Li, Yin, Zhang, & Wang, 2008; Meinert, Christiansen, Kristensen, Bjerregaard, & Aaslyng, 2008), and the results show that the breed greatly impacted pork flavour quality. In China, Tibetan and Sanmenxia pigs, as the local pig breeds, are well known for their favourable organoleptic properties and rich nutritional composition (Mi et al., 2019; Shen et al., 2014). As a typical hybrid pig, Duroc × (Landrace × Yorkshire) is now widely used for commercial production and the texture and flavour of this pork is notably different from that of Chinese local pig breeds (Lu, Li, Yin, Zhang, & Wang, 2008). The above studies were mainly focused on the physic-chemical and sensory characteristics in the different pork breeds, and it is unclear which flavour compounds are important for sensory attributes. Furthermore, due to the higher nutrition and flavour quality, Chinese local pork is usually at a higher price compared the typical hybrid pork. To obtain the higher profits, some people pass off the typical hybrid pork

as **Chinese local pork**. This behaviour results in economic loss to the meat industry and has negative effects on the reputation of **Chinese local pork**. Therefore, it is necessary to find a new method to identify **Chinese local pork**.

At present, a variety of analytical techniques have been employed for differentiation of meat in the scientific literature. Grunert, Stephan, Ehling-Schulz, and Johler (2016) provide a promising method to differentiate fresh and frozen/thawed chicken using Fourier transform infrared spectroscopy. Visible and near infrared spectroscopy technology can rapidly identify enhanced quality pork (Prieto, Juárez, Zijlstra, López-Campos, & Aalhus, 2015). Lopez-Oceja, Nuñez, Baeta, Gamarra, and Pancorbo (2017) reported that eight common meat species were identified using a high-resolution melt screening method. Additionally, our research team have reported an ICP-MS-based element profile (Mi, Shang, Jia, Zhang, & Fan, 2019) for the authentication of Taihe black-boned silky fowl. **To the best of our knowledge, most of the above analytical methods are widely used to distinguish different types of meat; however, there are only a few reports describing the use of chemometrics analysis of volatile flavour compounds to discriminate different breeds. A recent study has found that beef, pork and mixed (70% beef and 30% pork) minced meat could be easily discriminated and classified by a volatilomic approach based on volatile fingerprints (Pavlidis, Mallouchos, Ercolini, Panagou, & Nychas, 2019). Hence, volatiles analysis together with multivariate statistics is a promising approach for the differentiation of different varieties of pork.**

The aim of this study was to characterize the volatile profile in boiled pork from Tibetan, **Sanmenxia and Duroc × (Landrace × Yorkshire)**, and then to confirm the key odor-active compounds and potential flavour markers. Multivariate statistical methods for volatile compounds were used to explore the feasibility to differentiate boiled pork from **Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire) pigs**. The results of this study should provide a better understanding of the aroma characteristics of boiled pork and provide a novel strategy for the authentication of **boiled pork from Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire) pigs**.

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Highlight:

- A total of 61 volatile compounds were identified and quantified.
- 25 volatile compounds were considered as odour-active compounds.
- Boiled pork was clearly separated into three groups by chemometric analysis.
- The potential flavour markers were found in boiled pork of different breeds of pigs.
- GC-MS/O coupled with E-nose method is feasible to distinguish the different boiled pork.

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1 **Characterization and differentiation of boiled pork from Tibetan, Sanmenxia**
2 **and Duroc × (Landrac × Yorkshire) pigs by volatiles profiling and chemometrics**
3 **analysis**

4 Dong Han^{a,b}, Chun-Hui Zhang^{a,*}, Marie-Laure Fauconnier^b, Si Mi^a

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9 *University of Liege, 25030 Gembloux, Belgium*

10 **Corresponding author:**

11 *Chun-Hui Zhang, E-mail: dr_zch@163.com, Tel: 86-10-62819469

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1
2 13 **Abstract:** To characterize and differentiate boiled pork from three different breeds of
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4 14 pig (Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire)), the volatile
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7 15 compounds in each were analysed by gas chromatography-olfactometry-mass
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9 16 spectrometry (GC-MS/O) and electronic nose (E-nose) combined with chemometrics
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11 17 analysis. In total, 61 volatile compounds were identified, among which 25 compounds
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14 18 were selected as odour-active compounds in boiled pork. Moreover, seven
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16 19 odour-active compounds (hexanal, nonanal, 1-octen-3-ol, dimethyl disulphide,
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18 20 heptanal, 2-pentylfuran and 2-ethylfuran) were the main contributors to the integral
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20 21 flavour of boiled pork due to their higher odour activity values (OAVs) ranging from
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22 22 17.3-524.2. The odour-active compounds were examined by principal component
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24 23 analysis (PCA), agglomerative hierarchical clustering (AHC) and partial least
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26 24 squares-discriminant analysis (PLS-DA). The results showed that boiled pork from
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28 25 the three pig breeds could be clearly distinguished, and twelve odour-active
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30 26 compounds, including (*E,E*)-2,4-decadienal, ethyl hexanoate, dimethyl disulphide,
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32 27 hexanal, 2-acetylthiazole, (*E*)-2-nonenal, 1-octen-3-ol, (*E,E*)-2,4-nonadienal, heptanal,
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34 28 (*E*)-2-octen-1-ol, styrene and (*E*)-2-octenal, were determined as potential flavour
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36 29 markers for discrimination. This study indicated that GC-MS/O and E-nose with
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38 30 chemometrics analysis are feasible methods to characterize and discriminate boiled
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40 31 pork from three pig breeds.

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43 32 **Keywords:** GC-MS/O; E-nose; pork breeds; odour-active compounds; potential
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50 34 **Chemical compounds studied in this article:**
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2 35 Hexanal (PubChem CID: 6184)
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4 36 Nonanal (PubChem CID: 31289)
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9 38 Dimethyl disulphide (PubChem CID: 12232)
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16 41 2-Ethylfuran (PubChem CID: 18554)
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19 42 (*E,E*)-2,4-Decadienal (PubChem CID: 5283349)
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21 43 2-Acetylthiazole (PubChem CID: 520108)
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24 44 **1. Introduction**

27 45 According to a United States Department of Agriculture (USDA) report, global
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29 46 pork production is expected to rise to 113.0 million tons. China has the largest pork
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31 47 share in the global market, accounting for 48.7% (55.0 million tons) of total
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33 48 production in the whole world. Moreover, pork is popular with consumers due to its
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35 49 sensory attributes, such as tender texture, rich nutritional composition (Purriños,
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37 50 Franco, Carballo, & Lorenzo, 2012; Sivakumar 2016) and unique flavour (Straadt,
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39 51 Aaslyng, & Bertram, 2013). Flavour is one of the most important sensory attributes
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41 52 for consumers' ~~choice of~~ to judge the quality of pork (Wang, Song, Zhang, Tang, &
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43 53 Yu, 2016) and mainly associated with the generation of volatile compounds (Zhao et
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45 54 al., 2017). ~~So far,~~ Previous studies have indicated that over 1000 volatile compounds
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47 55 have been identified in meat and meat products, including aldehydes, ketones,
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57 compounds (Shahidi 1998). These compounds are mainly derived from a complex
58 series of chemical reactions (e.g., lipid oxidation, the Maillard reaction and
59 lipid-Maillard interactions) between precursors, intermediate reaction products and
60 degradation products (Jayasena, Ahn, Nam, & Jo, 2013).

61 To explore the composition, origin and formation of volatile compounds in
62 different pork products, many studies have been performed in recent years. A total of
63 149 volatile compounds (25 aldehydes, 18 phenols, 12 alcohols, 16 terpenes, 27
64 aromatic hydrocarbons, 18 aliphatic hydrocarbons, 17 ketones, 9 esters and 7 acids)
65 were identified from dry-cured hams using four different processing methods, among
66 which aldehydes and phenols were the more abundant volatiles (Petričević, Radovčić,
67 Lukić, Listeš, & Medić, 2018). The volatile compounds in six dry-cured meat
68 products were detected using a GC/MS technique; these compounds were then used to
69 identify the possible source of the typical volatiles (Domínguez et al., 2019). Due to
70 lipid oxidation, brine permeation and carbohydrate fermentation, the levels of volatile
71 compounds under high pressure treatment contributed were more than 70% of the
72 typical aroma, except for acetic acid (Yang, Sun, Pan, Wang, & Cao, 2018). Although
73 a large number of volatile compounds associated with different processing
74 technologies for specific types of pork have been fully analysed, information on
75 volatile profiles in different varieties of processed pork products are still lacking.
76 ~~Aroma compounds such as pyrazines, pyridines and furans in fried bacon and fried~~
77 ~~pork loin are thought to be responsible for the meaty aromas (Cross & Ziegler 2006;~~
78 ~~Timón, Carrapiso, Jurado, & van, 2004). A total of 38 volatiles were determined in~~

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2 79 raw ham using the HS-Trap GC-MS method (Bosse, Wirth, Konstanz, Becker, Weiss,
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4 80 & Gibis, 2017). A total of 149 volatile compounds of dry-cured hams from four
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7 81 different processing methods were identified and 15 of them were quantified
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9 82 (Petricevic, Marusic, Luki, Listes, & Medic, 2018). The above studies have mainly
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11 83 focus on the qualitative and quantitative analysis of volatile profiles of the pork from
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14 84 different processing technologies. However, there is a small number of studies
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17 85 regarding the volatile profiles of boiled pork of different breeds of pigs.

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20 86 As reported, the intramuscular fat, colour and flavour of pork from different pig
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22 87 breeds have been studied by many researchers (Lee et al. 2012; Lu, Li, Yin, Zhang, &
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27 89 the results show that the breed greatly impacted pork flavour quality. In China,
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29 90 Tibetan and Sanmenxia pigs, as the local pig breeds, are well known for their
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32 91 favourable organoleptic properties and rich nutritional composition (Mi et al., 2019;
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34 92 Shen et al., 2014). As a typical hybrid pig, Duroc × (Landrace × Yorkshire) is now
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41 95 2008). The above studies were mainly focused on the physic-chemical and sensory
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43 96 characteristics in the different pork breeds, and it is unclear which flavour compounds
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45 97 are important for sensory attributes. The cooked pork from Chinese indigenous breed
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47 98 pigs have higher flavor intensity than hybrid pigs (Lu, Li, Yin, Zhang, & Wang, 2008).
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49 99 In this study, both Tibetan pigs and Sanmenxia pigs belong to Chinese native breeds,
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52 100 Duroc × (Landrace × Yorkshire) pigs belong to hybrid pigs. Furthermore, Due due to
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2 101 the higher nutrition and flavour quality, ~~(Yang et al. 2014; Zhang et al. 2014)~~ Chinese
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4 102 local pork, the pork of Tibetan pigs was ~~is~~ usually at a higher price compared the
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6 103 typical hybrid pork. To obtain the higher profits, some people pass off the typical
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8 104 hybrid pork as Chinese local pork ~~Tibetan pork~~. This behaviour results in economic
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10 105 loss to the meat industry and has negative effects on the reputation of Chinese local
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12 106 pork ~~Tibetan pork~~. Therefore, it is necessary to find a new method to identify Chinese
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14 107 local pork ~~the pork of Tibetan pigs~~.

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19 108 At present, a variety of analytical techniques have been employed for
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21 109 differentiation of meat in the scientific literature. Grunert , Stephan, Ehling-Schulz,
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23 110 and Jöhler (2016) provide a promising method to differentiate fresh and
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25 111 frozen/thawed chicken using Fourier transform infrared (~~FTIR~~) spectroscopy. Visible
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27 112 and near infrared spectroscopy (~~Vis-NIRS~~) technology can rapidly identify enhanced
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31 114 Nuñez, Baeta, Gamarra, and Pancorbo (2017) reported that eight common meat
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33 115 species were identified using a high-resolution melt (~~HRM~~) screening method.
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35 116 Additionally, our research team have reported ICP-MS-based element profile (Mi,
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37 117 Shang, Jia, Zhang, & Fan, 2019) for the authentication of Taihe black-boned silky
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39 118 fowl. To the best of our knowledge, most of the above analytical methods are widely
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45 121 discriminate different breeds. A recent study has found that beef, pork and mixed (70%
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47 122 beef and 30% pork) minced meat could be easily discriminated and classified by a
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2 123 volatilomic approach based on volatile fingerprints (Pavlidis, Mallouchos, Ercolini,
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4 124 Panagou, & Nychas, 2019). Hence, volatiles analysis together with multivariate
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7 125 statistics is a promising approach for the differentiation of different varieties of
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9 126 pork. However, there is few researches regarding the discrimination method of
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11 127 different breeds of pigs though chemometrics analysis of volatile flavor compounds.
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14 The aim of this study was to characterize the volatile profile in boiled pork from
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16 129 Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire), different breeds of pigs and
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19 130 then to confirm the key odor-active compounds and potential flavour markers.

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21 131 Multivariate statistical methods for volatile compounds were used to explore the
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24 132 feasibility to differentiate boiled pork from Tibetan, Sanmenxia and Duroc ×
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26 133 (Landrace × Yorkshire) pigs different breeds of pigs. The results of this study should
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29 134 provide a better understanding of the aroma characteristics of boiled pork and provide
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31 135 a novel strategy for the authentication of boiled pork from Tibetan, Sanmenxia and
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33 136 Duroc × (Landrace × Yorkshire) pigs the different breeds of pork.
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36 137 **2. Materials and methods**

37 38 39 138 *2.1. Materials and chemicals*

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42 139 A total of 18 pigs from three breeds, including Tibetan pigs (n = 6, aged 5-6
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44 140 months), Sanmenxia pigs (n = 6, aged 5-6 months) and Duroc × (Landrac × Yorkshire)
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46 141 (n = 6, aged 5-6 months) were studied. Tibetan pigs (TB) was provided by Tibet Woye
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49 142 Tibetan Pig Development Co. Ltd. (Nyingchi, Tibet Autonomous Region, China).
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51 143 Sanmenxia pigs (SMX) and Duroc × (Landrace × Yorkshire) pigs (DLY) were
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54 144 obtained from Chuying Agro-Pastoral Group Co. Ltd. (Zhengzhou, Henan Province,
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2 145 China). All the pigs were reared under the same conditions and provided with the
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4 146 same feed. They were slaughtered following the same commercial procedures in the
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7 147 nearby abattoir. After cooling at 0-4°C for 24 h, *Triceps brachii* and *Biceps femoris*
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9 148 muscles of all of the pigs were dissected from the carcasses. Tibetan, SMX and DLY
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11 149 pork samples (n = 6 for each breed) from two different muscle were collected, and the
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14 150 same muscle gathered from two individual pigs of the same breed was combined as
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17 151 one sample (n = 3 for each muscle) for volatiles analysis. All pork samples were
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19 152 placed into ice-boxes and sent to the laboratory of Chinese Academy of Agricultural
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21 153 Sciences, Beijing. The study procedures were approved by the Animal Care and Use
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24 154 Committee of the Institute of Food Science and Technology, Chinese Academy of
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26 155 Agricultural Sciences, and performed in accordance with animal welfare and ethics.

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29 156 C₇-C₃₀ saturated alkanes (1000 µg/mL for each component in hexane) and
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31 157 2-methyl-3-heptanone (99%) were purchased from Sigma-Aldrich (Shanghai, China).

32 33 34 158 2.2. *Boiled pork muscles pretreatment*

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37 159 The skin, visible fat and connective tissues were removed from the pork of TB,
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39 160 SMX and DLY. Approximately 200 g of meat supplemented with 1.0% sodium
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42 161 chloride (based on the raw meat weight) and 150% (w/w) tap water were boiled in a
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44 162 low-density polyethylene bag. The pork samples were first heated from room
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47 163 temperature (22.3 ± 0.5°C) to a core temperature (80.0 ± 0.5°C), then held for 30 min.
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49 164 The boiled pork was cut into 1.0 × 1.0 × 1.0 cm³ cubes, ground with a pulveriser in
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51 165 liquid nitrogen and stored in a frozen state (-18°C) until use.
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2 166 2.3. *Solid-phase micro-extraction (SPME) of volatile compounds*
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5 167 The extraction method was modified from a previous study (Wang, Song, Zhang,
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7 168 Tang, & Yu, 2016). The volatile compounds of boiled pork were extracted using a
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9 169 manual SPME equipped with a 50/30 μm
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11 170 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco,
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13 171 Inc., Bellefonte, PA, USA). The pork sample was precisely weighed—5.00 g—and
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15 172 transferred to a 40 ml vial. Thereafter, 2-methyl-3-heptanone was dissolved in hexane
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17 173 as an internal standard solution to a final concentration of 0.41 mg/ml, 1 μl of this
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19 174 solution was added and the vial was tightly capped with Teflon/silicon septum. The
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21 175 vial was equilibrated at 60°C for 20 min in a water bath. The selected fibre was
22
23 176 exposed to the headspace of the samples to absorb the volatile compounds for 40 min
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25 177 at 60°C. Upon completion, the fibre was inserted into the injection port (250°C) of the
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27 178 GC instrument to desorb the analyses for 5 min. All samples were extracted in
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29 179 triplicate.
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36 180 2.4. *GC-MS/O analysis*
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39 181 The method was performed according to the method of Liu, He, and Song (2018)
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41 182 with minor modifications. Analyses of volatile compounds were performed out on an
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43 183 Agilent 7890A gas chromatograph coupled with an Agilent Model 7000B series mass
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45 184 spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA). The GC-MS
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47 185 system was equipped with an olfactory detector port (Sniffer 9000; Brechbuhler,
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49 186 Schlieren, Switzerland). The volatiles were separated on polar DB-wax and non-polar
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51 187 DB-5 capillary column (30 m \times 0.32 mm i.d., 0.25 μm film thickness; J & W
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2 188 Scientific, Inc., Folsom, CA, USA). Ultra-high purity helium ($\geq 99.999\%$) was used as
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4 189 the carrier gas and the constant flow rate was 1.2 ml/min. Temperature programme
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7 190 began with isothermal heating at 40°C for 3 min, then rising to 200°C at a rate of
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9 191 5°C/min, followed by another increase to 230°C (DB-wax) and 250°C (DB-5) at
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11 192 10°C/min. Final temperature was held for 3 min. The transfer line temperatures were
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13 193 maintained at 240°C (DB-wax) and 270°C (DB-5). The effluent from the capillary
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15 194 column was split 5:1 (v/v) between the mass spectrometry detector and the olfactory
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17 195 detector port. Electro-impact mass spectra were generated at 70 eV with an m/z scan
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19 196 range from 50 to 400 amu. The ion source temperature was 230°C. A panel that
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21 197 contains eight trained staff was utilized for the sniffing test on the GC-O. Humidified
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23 198 air was supplied to the sniff port with a flow of 30 ml/min to avoid dryness of the
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25 199 nasal mucosa.

31 200 2.5. Identification and quantification of volatile compounds

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34 201 The volatile components were identified by comparing their electron ionization
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36 202 (EI) spectra with the database records provided by the National Institute of Standards
37
38 203 and Technology (NIST) Mass Spectral Library (Version 2.0), GC retention indices
39
40 204 (RIs) and odour descriptions described in the literature and in online databases
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42 205 (<http://www.flavornet.org>; <http://www.odour.org.uk>).

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45 206 Quantitative analysis of the volatile compounds was performed using a
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47 207 calibration method with an internal standard (Zhou, Chong, Ding, Gu, & Liu, 2016).
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49 208 The concentrations of the volatile constituents were measured by the calibration
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51 209 curves of the GC-peak area and the amount ratios for the target analyte relative to
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2 210 2-methyl-3-heptanone. The final results were expressed as μg volatile compounds/kg
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4 211 of the boiled pork. Each value represented the average of triplicate determinations.

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7 212 2-methyl-3-heptanone was used as the internal standard without considering the
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9 213 calibration factors, that is, all calibration factors were considered to be 1.00. The
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11 214 involved equation can be written as follows:

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$$\text{Conc} \left(\frac{\mu\text{g}}{\text{kg}} \right) = \frac{\text{Peak area ratio}(\text{volatile}/\text{IS}) \times 0.41 \mu\text{g}(\text{IS})}{5 \text{ g}(\text{boiled pork})} \times 1000$$

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17 216 OAVs were calculated according to the method of Liu, He, and Song (2018) using the
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19 217 following addition equation:

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$$\text{OAV}_i = \frac{C_i}{\text{OT}_i}$$

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25 219 where C_i is the concentration of the compound in the boiled pork and OT_i is the odour
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27 220 threshold in water. OT_i was obtained from the online database
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29 221 (<http://www.odour.org.uk>) and some references related to flavour. Compounds with
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31 222 and $\text{OAV} \geq 1$ were considered to be the main contributors to total flavour.

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36 223 *2.6. E-nose analysis*

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38 224 In this study, the odour profile of different boiled pork samples was
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41 225 discriminated using a portable electronic nose (PEN3) that operates with an
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43 226 enrichment and desorption unit (EDU) from Win Muster Airsense Analytics, Inc.
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45 227 (Airsense, Germany). This instrument consisted of a sampling apparatus, a detector
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47 228 unit that contains ten metal oxide sensors (Gao, Liu, An, Zhang, Ma, & Cui, 2017),
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49 229 and pattern identification software for data recording and elaboration (Wang, Wang,
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51 230 Liu, & Liu, 2012). Table 1 lists all sensors and their major applicants.
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231 Approximately 1.00 g of a boiled pork sample was added to a 10 ml glass vial. A
232 filtered and dried air flow (99%, 300 ml/min) was used as a carrier gas for E-nose
233 detection. The data acquisition period lasted for 60 s, and an additional 180 s was
234 required for system rebalance. For each sample, the E-nose analysis was repeated
235 three times for the same conditions.

236 *2.7. Statistical analysis*

237 The contents of all volatile compounds and OAVs of the odour-active
238 compounds were performed using one-way analysis of variance (ANOVA) and
239 Duncan's multiple range tests in the SPSS software (v. 19.0, SPSS, Inc., Chicago, IL,
240 USA). The significance level was set at $P < 0.05$. Principal component analysis
241 (PCA), agglomerative hierarchical clustering (AHC) and partial least
242 squares-discriminant analysis (PLS-DA) were performed based on the odour-active
243 compounds (OAV > 1) using the software XLSTAT (2016) from Addinsoft
244 (Barcelona, Spain). The odour-active compounds with variable importance in the
245 projection (VIP) score > 1 in the PLS-DA analysis and a p-value < 0.05 in the
246 ANOVA were considered significantly different among all boiled pork samples.
247 E-nose linear discriminate analysis (LDA) was conducted using the *WinMuster*
248 software (version 1.6, Airsense Analytics, Schwerin, Germany) to differentiate the
249 boiled pork samples according to the overall flavour.

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2 250 **3. Results and discussion**

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5 251 *3.1. Volatile profiling of boiled pork by GC-MS/O*

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8 252 *3.1.1. Volatile composition of boiled pork*

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10 253 A total of 61 volatile components were identified in boiled pork from the *Triceps*
11
12 254 *brachii* and *Biceps femoris* muscles from different pig breeds by SPME-GC-MS/O, as
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15 255 is shown in Table 2. These compounds can be classified into nine chemical families,
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18 256 including aldehydes (50.5%-65.7%, 25/61), alcohols (4.8%-10.3%, 7/61), ketones
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20 257 (0.4%-0.9%, 1/61), esters (2.8%-12.3%, 3/61), aromatics (0.6%-8.4%, 8/61),
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23 258 hydrocarbons (2.1%-5.7%, 8/61), furans (5.3%-7.7%, 3/61), N-containing compounds
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25 259 (3.9%-6.5%, 2/61) and S-containing compounds (4.0%-9.5%, 4/61). The results
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28 260 showed that most of these compounds have been reported in the three different pig
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30 261 breeds (Pan, Yang, Zhu, and Wu, 2014). Among them, the largest number of
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33 262 aldehydes were found in boiled pork, followed by hydrocarbons, and aromatic
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35 263 compounds. Moreover, aldehyde compounds, which accounted for greater than 50.0%
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37 264 of the total volatile compounds, were the most abundant in boiled pork.

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40 265 The concentration ratios and quantities of each group of volatile compounds in
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43 266 boiled pork is presented in Table 2. For the pig breeds yielding the boiled pork, there
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45 267 were 52, 44 and 54 volatile compounds in DLY, SMX and TB, respectively. The
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47 268 proportions of aldehydes and ketones (64.2%-65.7% and 0.5%-0.9%, respectively)
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50 269 were highest in TB, and the ratios of ethers, furans and S-containing compounds
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52 270 (9.0%-12.3%, 6.7%-7.7% and 8.6%-9.5%, respectively) were the highest in SMX. In
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2 271 contrast, aromatic compounds (7.7%-8.4%) were the most abundant in DLY. Owing
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4 272 to the main flavour of pork from aldehydes, furans and S-containing compounds and
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6 273 their presence in TB and SMX, TB and SMX had significant contributions to overall
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8
9 274 flavour. This result is in accordance with the study of Zhao et al., (2017). With respect
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11 275 to the parts for boiled pork, the major volatile components in the *Triceps brachii* and
12
13 276 *Biceps femoris* muscles of DLY were aldehydes, alcohol and aromatics, which
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15 277 accounted for total concentrations of 55.1%-56.5%, 8.6%-10.3% and 6.6%-8.8%,
16
17 278 respectively. The abundant volatiles in *Triceps brachii* and *Biceps femoris* muscles
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19 279 from SMX were aldehydes, ethers and S-containing compounds, which maintained
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21 280 the relationship of aldehydes > ethers > S-containing compounds. The main volatile
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23 281 compounds in *Triceps Brachii* and *Biceps Femoris* from TB were aldehydes
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25 282 (64.2%-65.7%) and S-containing compounds (6.2%-8.1%). These analyses concluded
26
27 283 that aldehydes and S-containing compounds had a dominant role in cooked pork
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29 284 (Aaslyng & Meinert 2017).
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36 285 Qualitative and quantitative analyses of the volatile components in boiled pork
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38 286 from different pig breeds are listed in Table 3. Aldehyde compounds, similar to the
39
40 287 important volatile compounds in all types of meat products, were produced primarily
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42 288 by lipid oxidation and degradation reactions. Strecker degradation products of amino
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44 289 acids (Zhao et al., 2017; Li, Li, Zhang, Wang, Tang, and Chen, 2016) are also known
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46 290 to be major contributors to the unique flavour of cooked pork due to their low odour
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48 291 threshold (Lorenzo & Fonseca 2014). In this study, aldehydes were the most abundant
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50 292 groups and had the highest number of compounds in boiled pork samples. Eight of
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2 293 these compounds were simultaneously detected in all the boiled pork samples,
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4 294 including four alkenals (hexanal, heptanal, nonanal and hexadecanal), two alkadienals
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7 295 ((*E*)-2-octenal and (*E*)-2-nonenal) and two phenyl-containing aldehydes
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9 296 (benzaldehyde and 4-ethylbenzaldehyde). The four alkenals and two alkadienals are
10
11 297 unsaturated fatty acid degradation products (Karahadian & Lindsay 1989). Meanwhile,
12
13 298 the two phenyl-containing aldehydes are usually derived from the Strecker reaction
14
15 299 (MacLeod, Ames, & Betz, 1988). Hexanal was the most abundant aldehyde and
16
17 300 presented grassy notes, while (*E*)-2-octenal had a low odour threshold ($3 \mu\text{g}\cdot\text{kg}^{-1}$) and
18
19 301 was described as having fatty notes (Gu, Wang, Tao, & Wu, 2013; Wang et al., 2018).
20
21
22 302 Moreover, the hexanal and (*E*)-2-octenal contents in DLY were significantly higher
23
24 303 ($P < 0.01$) than in SMX and TB, indicating that the extent of lipid oxidation in DLY
25
26 304 was greater. The nonanal and benzaldehyde contents in TB were significantly higher
27
28 305 ($P < 0.001$) than in DLY and SMX. This showed that TB had an advantage in the
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30 306 contribution to fruity and floral notes. Additionally, (*E,E*)-2,4-heptadienal and
31
32 307 9,12,15-octadecatrienal were exclusively found in boiled pork from SMX and TB and
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34 308 promoted a sweeter and fruit aroma (Allen & Hamilton 1989).
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40 309 Alcohols are mainly generated by the oxidative decomposition of lipids (Zou,
41
42 310 Kang, Liu, Qi, Zhou, & Zhang, 2018). Compared with short straight chain alcohols,
43
44 311 long chain alcohols are considered to have more contributions to the aroma of meat
45
46 312 products due to their lower odour thresholds (Li, Li, Zhang, Wang, Tang, & Chen,
47
48 313 2016). Seven alcohols were detected in this study, including three straight chain
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50 314 alcohols (1-pentanol, 1-hexanol and 1-octanol) and four branched chain alcohols
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2 315 (1-octen-3-ol, 2-hexyldecanol, (*E*)-2-octen-1-ol and anethole). Among these volatile
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4 316 compounds, 1-octen-3-ol, 1-octanol and (*E*)-2-octen-1-ol were found in all three
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6
7 317 varieties of boiled pork. The average contents of 1-octen-3-ol, with mushroom notes,
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9 318 and (*E*)-2-octen-1-ol, with green apple notes, in boiled pork from DLY were
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11 319 significantly ($P < 0.01$) higher than those from TB and SMX. Moreover,
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14 320 2-hexyldecanol was only present in DLY, which indicated that it contributes more
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16 321 pleasant fruity and floral aromas (Petričević, Radovčić, Lukić, Listeš, & Medić, 2018)
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18
19 322 to overall flavour.

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21 323 Furan, nitrogen and sulphur-containing compounds are well known as important
22
23 324 heterocyclic compounds in meat products (Wang et al., 2018). Among the three furan
24
25 325 compounds, 2-pentylfuran, with a fruity and buttery odour, had the highest contents
26
27 326 ($108.5\text{-}244.0 \mu\text{g}\cdot\text{kg}^{-1}$) in all the boiled pork, which could be due to linoleic acid
28
29 327 oxidization (Aparicio, Morales, & Alonso, 1996). 2-ethylfuran and 2-furanmethanol
30
31 328 usually have pungent and caramel odours and have been reported in cooked meat (Gu,
32
33 329 Wang, Tao, & Wu, 2013; Yang, Pan, Zhu, & Zou, 2014). For two nitrogen-containing
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35 330 compounds, the contents of pyridine and 2-acetylpyrazine were significantly higher
36
37 331 ($P < 0.01$) in boiled meat from pig *Triceps brachii* muscle than in the boiled meat
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39 332 from pig *Biceps Femoris* muscle. Furthermore, 3-methylthiophene and benzothiazole
40
41 333 were very abundant in the boiled meat from pig *Biceps Femoris* muscle, while the
42
43 334 amounts of dimethyl disulphide and 2-acetylthiazole identified in TB were greater
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45 335 than in DLY and SMX. The comparative analysis indicated that these four
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47 336 sulphur-containing compounds in boiled pork of *Biceps Femoris* muscle in TB are
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2 337 regarded as the major contributor to the cooked cabbage and roasted flavours (Zhou,
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4 338 Chong, Ding, Gu, & Liu, 2016). Previous reported noted that these compounds might
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7 339 originate from sulphur amino acids (free, peptidic and proteinic amino acids),
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9 340 thiamine or glutathione (Girard & Durance, 2010).

11
12 341 Eight aromatic hydrocarbons and eight aliphatic hydrocarbons were identified in
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14 342 all the boiled pork samples. All of these compounds are usually formed by lipid
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16
17 343 oxidation (Kang, Gao, Ge, Zhou, & Zhang, 2017). Hydrocarbons had few effects on
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19 344 the aromatic profiles of meat products due to their high odour thresholds (Qi, Liu,
20
21 345 Zhou, & Xu, 2017). Compared to boiled pork from SMX and TB, there were more
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24 346 and greater amounts of hydrocarbons in boiled pork from DLY, which may be due to
25
26 347 the higher levels of lipid oxidation. Ester compounds can be formed by the
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29 348 esterification of acids and alcohols. A previous study showed that short-chain esters
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31 349 have fruity notes and long-chain esters have fatty notes (Wang et al., 2018). Terpinyl
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34 350 acetate and ethyl hexanoate were only found in SMX and may be used to distinguish
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36 351 boiled pork from different pig breeds. Vinyl hexanoate was present in all the
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39 352 investigated boiled pork samples.

41 353 *3.1.2. Odour-active compounds in boiled pork*

44 354 The odour-active compounds in this study were defined as the compounds with
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47 355 OAVs > 1. The OAVs of odour-active compounds in boiled pork are presented in
48
49 356 Table 4. Statistical analysis showed that the OAVs of 25 volatile compounds showed
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51 357 significant differences ($P < 0.05$) in boiled pork from the three pig breeds. Among the
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54 358 three varieties of boiled pork, TB contained the largest number of the odour-active
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2 359 constituents, including fourteen aldehydes, three alcohols, one hydrocarbon, two
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4 360 furans, one N-containing compound and three S-containing compounds (total of
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6
7 361 twenty-five), which indicated that TB displayed the most overall flavour among the
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9 362 meat samples. Compared with *Triceps Brachii* muscle from TB, there was a greater
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11 363 variety of odour-active compounds in *Biceps Femoris* muscle from TB. SMX
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13 364 contained 22 aroma-active constituents, which was the fewest odour-active
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15 365 compounds among the boiled pork from the three different pig breeds. For boiled pork
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17 366 from both the *Triceps Brachii* and *Biceps Femoris* muscles, the OAVs of half of the
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19 367 odour-active compounds did not show significant differences ($P > 0.05$), indicating
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21 368 that the muscles (*Triceps Brachii* and *Biceps Femoris*) presented similar flavour
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23 369 characteristics. In a word, the breed was considered as the main influencing factor for
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25 370 the overall flavour of boiled meat.
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32 371 As shown in Table 4, the following seven odour-active constituents with
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34 372 relatively high OAVs were detected in all samples: hexanal (OAV at 213.9-524.2),
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36 373 nonanal (OAV at 248.7-454.6), 1-octen-3-ol (OAV at 56.9-194.3), dimethyl
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38 374 disulphide (OAV at 76.8-141.3), heptanal (OAV at 19.0-41.7), 2-pentylfuran (OAV at
39
40 375 18.1-40.7) and 2-ethylfuran (OAV at 17.3-22.9). These constituents were regarded as
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42 376 key odour-active compounds due to their significant contributions to the integral
43
44 377 flavour. Linear aldehydes such as hexanal, nonanal and heptanal, with grass and fatty
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46 378 notes (Petričević, Radovčić, Lukić, Listeš, & Medić, 2018), come from lipid oxidation
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48 379 and may contribute to the overall flavour. 1-Octen-3-ol was the only alcohol among
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50 380 the odour-active compounds and has been reported to be generated by β -oxidation
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381 (Girard & Durance, 2010). Two furans, namely, 2-pentylfuran and 2-ethylfuran,
382 might impart rubber and sweet flavours to the boiled pork, respectively. Dimethyl
383 disulphide, with cooked cabbage notes, is an important fraction of aroma in fish paste
384 products (Giri, Osako, & Ohshima, 2010).

385 *3.2. Discrimination of boiled pork by PCA, PLS-DA and AHC*

386 To better visualize the data and reduce the dimensions of the original variables,
387 PCA was performed to discriminate the boiled pork from the *Triceps Brachii* and
388 *Biceps Femoris* muscles in the three pig breeds. Twenty-five odour-active compounds
389 (OAVs > 1) were analysed by PCA. PCA scoring and a loading plot are presented in
390 Fig. 1. The first two principal components account for 34.94% and 27.81% of the
391 variance, respectively, (62.76% in total). The six sample groups are clearly well
392 discriminated from one another. A clear separation between SMX and DLY can be
393 observed for PC1, while TB was significantly different from SMX and DLY with
394 respect to PC2. As shown in Fig. 1 and Table 5, nine aldehydes (hexanal, $r=0.727$;
395 heptanal, $r=0.740$; (*E*)-2-octenal, $r=0.945$; (*E*)-2-nonenal, $r=0.617$;
396 (*E,E*)-2,4-nonadienal, $r=0.846$; (*E,E*)-2,4-decadienal, $r=0.790$; 1-octen-3-ol, $r=0.747$
397 and (*E*)-2-octen-1-ol, $r=0.708$) and one hydrocarbon (styrene, $r=0.892$) had high
398 correlation coefficients with the positive side of PC1, which were present in DLY
399 with high OAVs (Table 3). In contrast, only 2-acetylthiazole ($r=-0.909$), 2-ethylfuran
400 ($r=-0.677$) and dimethyl disulphide ($r=-0.671$) showed high correlation coefficients
401 with the negative side of PC1. Moreover, PC2 on the positive axis was highly
402 influenced by benzaldehyde ($r=0.988$), nonanal ($r=0.923$), octanal ($r=0.791$), anethole

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2 403 (r=0.863) and (*E*)-2-nonenal (r=0.671), indicating that these compounds were the
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4 404 important odour-active compounds in TB (Fig. 1), while PC2 on the negative axis was
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7 405 highly influenced by ethyl hexanoate and (*E*)-2-octen-1-ol (r=-0.682). Hence,
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9 406 (*E*)-2-nonenal was highly associated with the PC1 and PC2 positive axis and
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11 407 (*E*)-2-octen-1-ol was highly associated with the PC1 positive and PC2 negative axes.
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14 408 However, 2-acetylpyrazine and decanal were lowly relevant to the plane (correlation
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16 409 coefficients < 0.400). This suggested that these two compounds could not be
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19 410 described by PC1 and PC2. Additionally, according to the VIP scores in the PLS-DA
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21 411 analysis and p-values in the ANOVA of the studied odour-active compounds (Table
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23 412 5), it could be concluded that a total of twelve volatile compounds with a VIP score >
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26 413 1 and p-value < 0.05 were considered as potential flavour markers for the
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29 414 differentiation of boiled pork. These odour-active compounds were
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31 415 (*E,E*)-2,4-decadienal, ethyl hexanoate, dimethyl disulphide, hexanal, 2-acetylthiazole,
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33 416 (*E*)-2-nonenal, 1-octen-3-ol, (*E,E*)-2,4-nonadienal, heptanal, (*E*)-2-octen-1-ol, styrene
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36 417 and (*E*)-2-octenal.

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38 418 AHC can be used to depict the similarities and differences among different
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41 419 boiled pork. Ward's method with a metric of Euclidean distance was applied in this
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43 420 study. The results as a dendrogram are presented in Fig. 2. The boiled meat samples
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45 421 were divided into three clusters. The third cluster included *Triceps Brachii* and *Biceps*
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47 422 *Femoris* muscles from SMX, with the lowest dissimilarity index, indicating that the
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50 423 *Triceps Brachill* and *Biceps Femoris* muscles from SMX have the most similar
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53 424 volatile profiles. Similarly, the second cluster, with *Triceps Brachii* and *Biceps*

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2 425 *Femoris* muscles from DLY, possessed similar volatile profiles. The first cluster
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4 426 consisted of *Triceps Brachii* and *Biceps Femoris* muscles from TB and had the
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7 427 highest dissimilarity index; this illustrated that the overall flavour of *Triceps Brachii*
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9 428 and *Biceps Femoris* muscles from TB was greatly different from that of the boiled
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11 429 pork from the other two pig varieties.

14 430 3.3. Volatile profiling of boiled pork using E-nose

17 431 The signal from 10 sensors in response to volatile compounds from different
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19 432 boiled pork are presented in Fig. 3. Fig. 3a-c show the that responses of all ten sensors
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21 433 to boiled pork from *Triceps Brachii* and *Biceps Femoris* muscles from the three pig
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23 434 breeds had no significant differences, which explained their similar flavour. This also
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25 435 showed that W5S (broad-range nitrous oxides), W1W (terpenes and
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27 436 sulphur-containing organic compound), and W2W (aromatics and organic sulphides)
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29 437 had higher responses than other sensors, which suggested that they may contain more
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31 438 heterocyclic compounds, such as furans and N- and S-containing compounds. The
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33 439 response values of W1C and W3C were less than one. Both sensors were mainly
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35 440 sensitive to aroma components and ammonia. As shown in Fig. 3d and e, the signals
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37 441 from the W5S and W1W sensors to the boiled pork from the three pig breeds
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39 442 obviously varied. Sensor W5S showed stronger responses to SMX, and while sensor
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41 443 W1W showed weaker responses to that breed. In a word, this result indicated that the
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43 444 influence of different pig breeds on flavour is greater than from different pig parts for
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45 445 boiled pork.

54 446 3.4. Discrimination of boiled pork by PCA and LDA

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447 E-nose analysis was performed to obtain a description of the odour profiles of
448 boiled pork from different pig parts and breeds, and the PCA analysis results are
449 shown in Fig. 4a. The plot consists of two axes showing PC1 and PC2, which could
450 explain 99.46% of the total variance. PC1 accounted for 94.83% and PC2 accounted
451 for 4.63%. The contribution variance of PC1 and PC2 is over 90%, indicating that the
452 first two PCs were sufficient to explain the maximum variation in the original types of
453 boiled pork. According to Fig. 4a, the dots corresponding to the *Triceps Brachii* and
454 *Biceps Femoris* muscles from SMX and DLY had some overlap on PC1, and the
455 sample points for the *Triceps Brachii* and *Biceps Femoris* muscles for TB were close
456 to one another. Thus, the boiled pork samples can be divided into three groups (SMX,
457 DLY and TB). This result illustrated that the boiled pork from different pig breeds
458 had significantly different flavours, and that boiled pork from the *Triceps Brachii* and
459 *Biceps Femoris* muscles of pigs had similar aroma compositions.

460 An LDA was also performed to investigate the similarities and differences
461 among the six sample groups. As shown in Fig. 4b, the first two PCs account for
462 88.58% of the variation in the data. Comparative analysis of all samples including
463 each boiled pork sample could be clearly discriminated using LDA. The sample
464 points for the *Triceps Brachii* and *Biceps Femoris* muscles from DLY and TB were
465 close on PC1 (72.58%) and PC2 (16.00%), respectively. Moreover, the SMX *Triceps*
466 *Brachii* and *Biceps Femoris* muscles clustered together on PC1 and PC2, which
467 demonstrated that the odour profiles of the boiled pork from the *Triceps Brachii* and
468 *Biceps Femoris* muscles of pigs appeared similar. The boiled pork from different pig

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2 469 breeds were far from one another. This demonstrated that there were considerable
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4 470 differences in their flavours. Both multivariate analyses (PCA and LDA) performed
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7 471 on E-nose data were suitable to distinguish the boiled pork based on its flavour
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9 472 profile.

12 473 **4. Conclusions**

15 474 In this study, a total of 61 volatile compounds were identified and quantified in
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17 475 boiled pork from different pig breeds by SPME-GC-MS/O. These compounds can be
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19 476 divided into nine categories: aldehydes, alcohols, ketones, esters, aromatics,
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22 477 hydrocarbons, furans, N- and S-containing compounds. The key odour-active volatiles
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24 478 from the evaluated samples were hexanal (OAV at 213.9-524.2), nonanal (OAV at
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27 479 248.7-454.6), 1-octen-3-ol (OAV at 56.9-194.3), dimethyl disulphide (OAV at
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29 480 76.8-141.3), heptanal (OAV at 19.0-41.7), 2-pentylfuran (OAV at 18.1-40.7) and
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32 481 2-ethylfuran (OAV at 17.3-22.9), which significantly contribute to the overall flavour.
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34 482 Moreover, according to multicomponent statistics analyses, including PCA, PLS-DA,
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37 483 AHC and LDA, the boiled pork from different pig breeds could be classified into
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39 484 three separate groups. Twelve odour-active compounds were confirmed as potential
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42 485 flavour makers for the differentiation of boiled pork among the three pig breeds.

44 486 Overall, it can be concluded that the characterization and differentiation of boiled
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47 487 pork from TB, SMX and DLY pigs by volatiles profiling and chemometrics analysis
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49 488 has the potential to be a feasible method to evaluate pork from different breeds.
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52 489 Moreover, further studies should include more pork samples to build a more reliable
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54 490 data model and validate key flavour compounds by aroma recombination and
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491 omission analysis.

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498 Zhang declares that he has no conflict of interest. Marie-Laure Fauconnier declares
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500 **Ethical Approval:** All applicable international, national, and/or institutional
501 guidelines for the care and use of animals were followed.

502 **Informed Consent:** Informed consent was obtained from all individual participants
503 included in the study.

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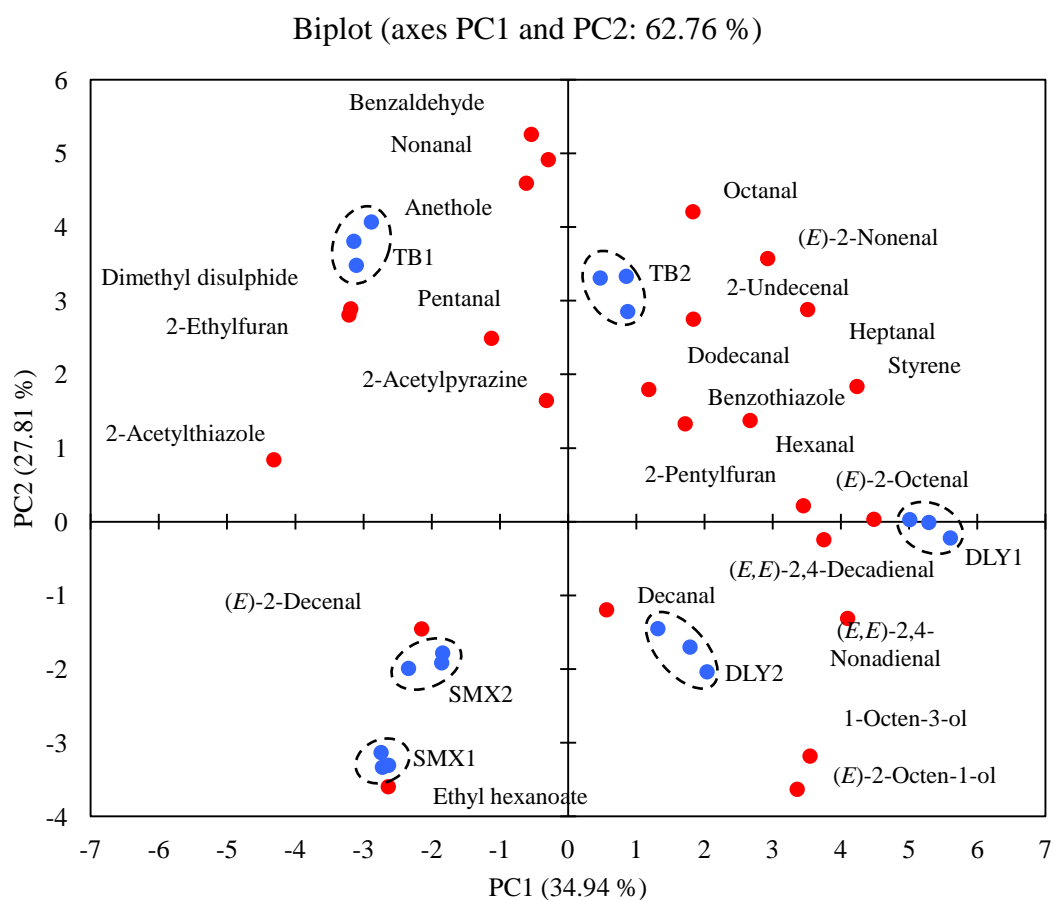


Fig. 1 PCA for odour-active compounds of the different boiled pork (TB1 = *Triceps Brachii* muscle of Tibetan pigs, TB2 = *Biceps Femoris* muscle of Tibetan pigs, DLY1 = *Triceps Brachii* muscle of Duroc × (Landrace × Yorkshire), DLY2 = *Biceps Femoris* muscle of Duroc × (Landrace × Yorkshire), SMX1 = *Triceps Brachii* muscle of Sanmenxia pigs, SMX2 = *Biceps Femoris* muscle of Sanmenxia pigs). The blue dots represent the samples from the different boiled pork, and the red dots represent odour-active compounds.

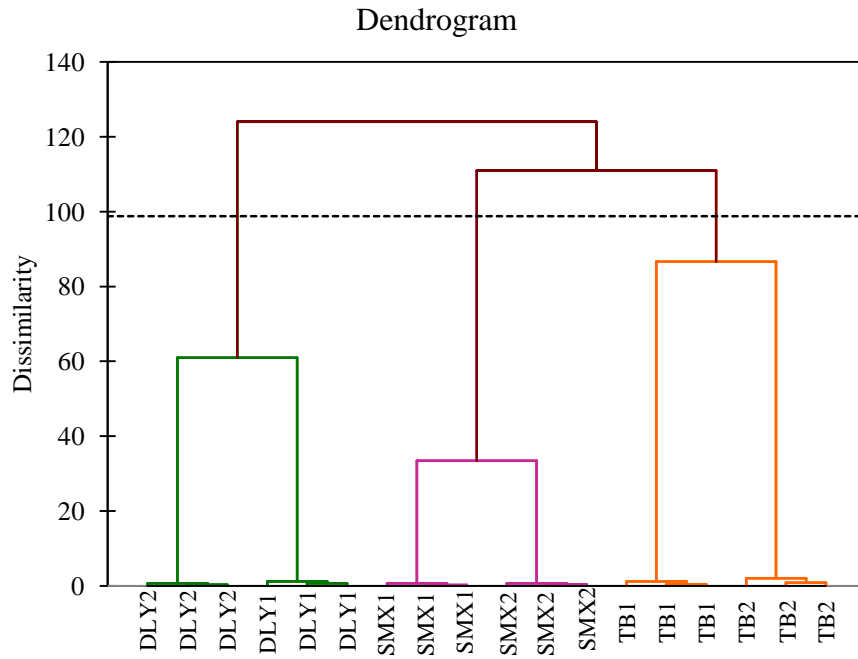
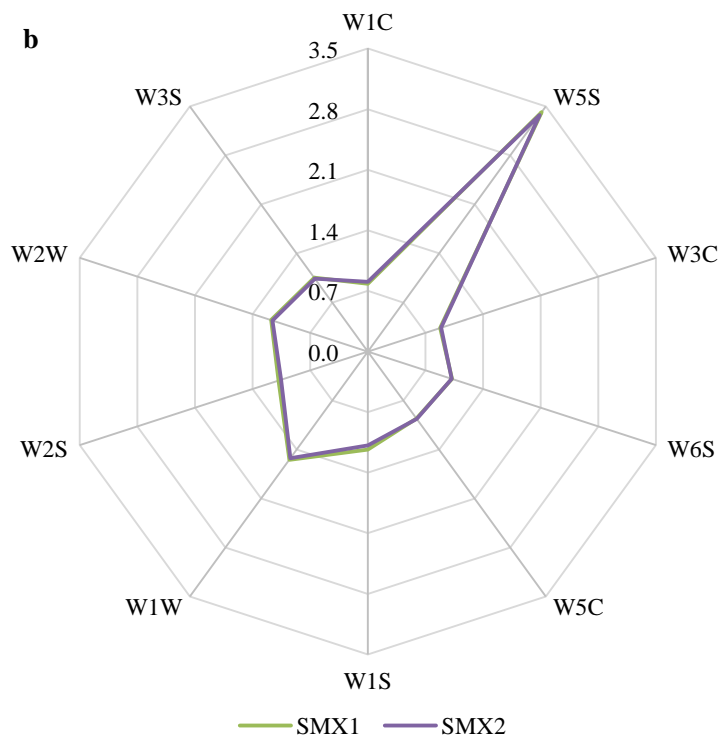
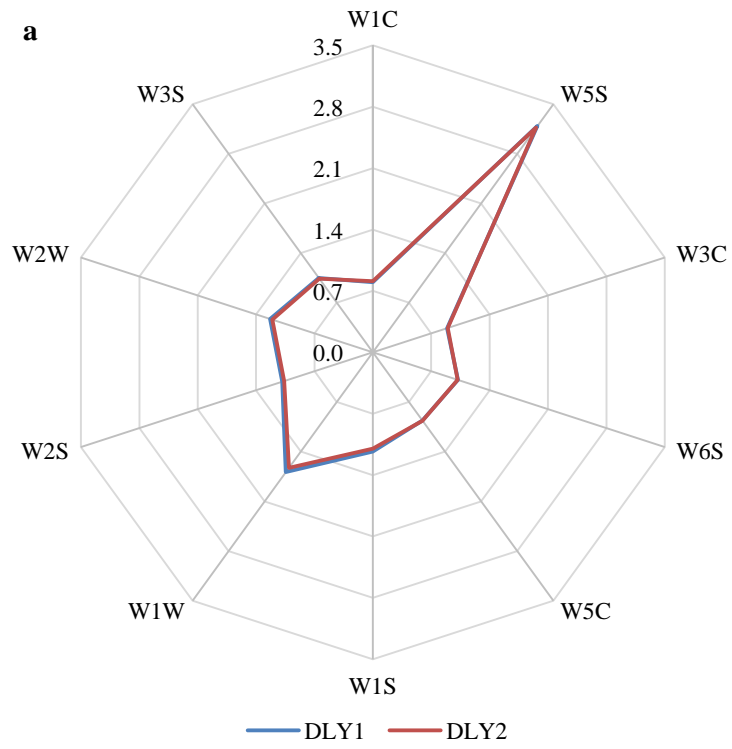
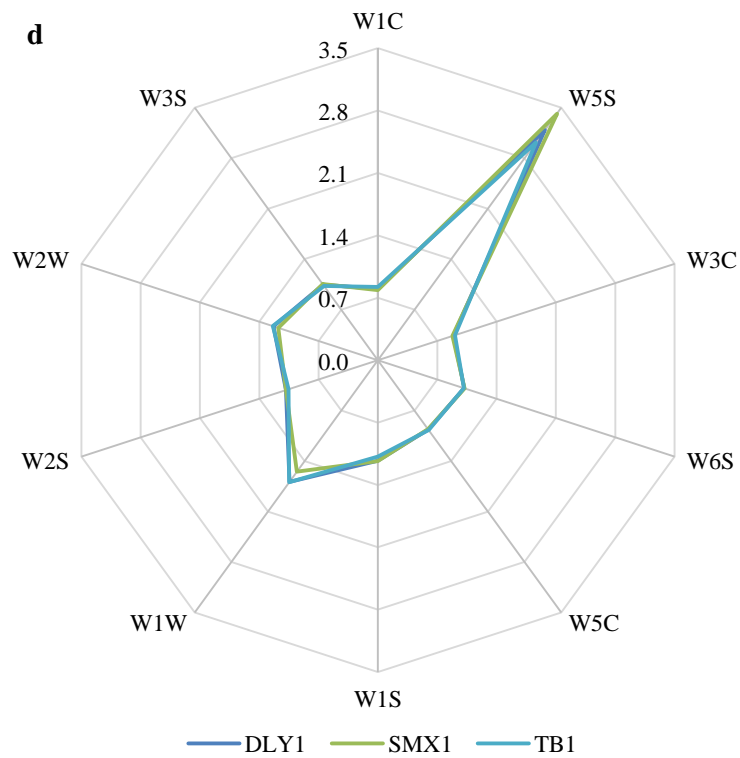
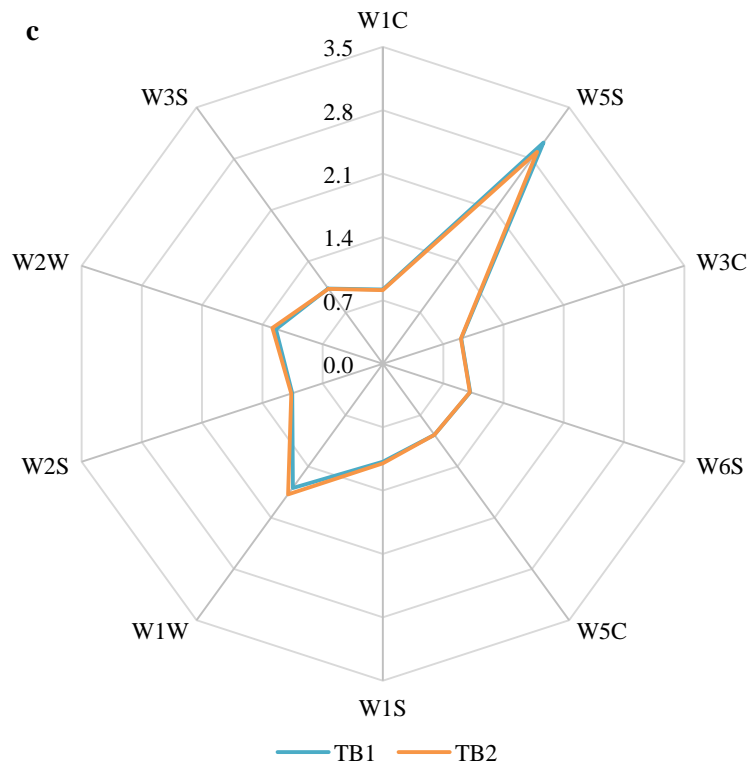


Fig. 2 AHC results of the different boiled pork (TB1 = *Triceps Brachii* muscle of Tibetan pigs, TB2 = *Biceps Femoris* muscle of Tibetan pigs, DLY1 = *Triceps Brachii* muscle of Duroc × (Landrace × Yorkshire), DLY2 = *Biceps Femoris* muscle of Duroc × (Landrace × Yorkshire), SMX1 = *Triceps Brachii* muscle of Sanmenxia pigs, SMX2 = *Biceps Femoris* muscle of Sanmenxia pigs).





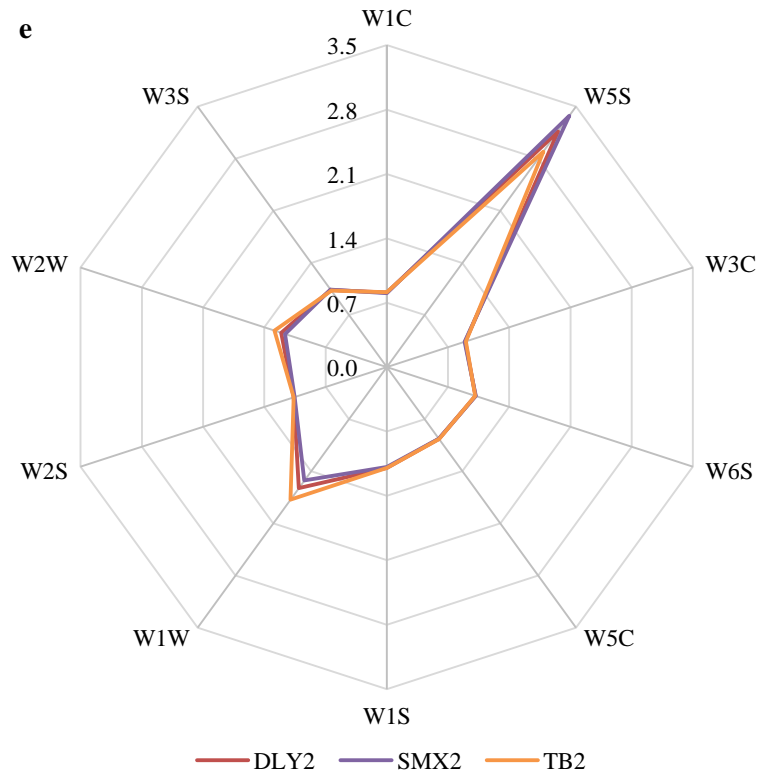


Fig. 3 Radar charts of E-nose data from DLY1 and DLY2 (a), SMX1 and SMX2 (b), TB1 and TB2 (c), DLY1, SMX1 and TB1 (d), DLY2, SMX2 and TB2 (e) (TB1 = *Triceps Brachii* muscle of Tibetan pigs, TB2 = *Biceps Femoris* muscle of Tibetan pigs, DLY1 = *Triceps Brachii* muscle of Duroc × (Landrace × Yorkshire), DLY2 = *Biceps Femoris* muscle of Duroc × (Landrace × Yorkshire), SMX1 = *Triceps Brachii* muscle of Sanmenxia pigs, SMX2 = *Biceps Femoris* muscle of Sanmenxia pigs).

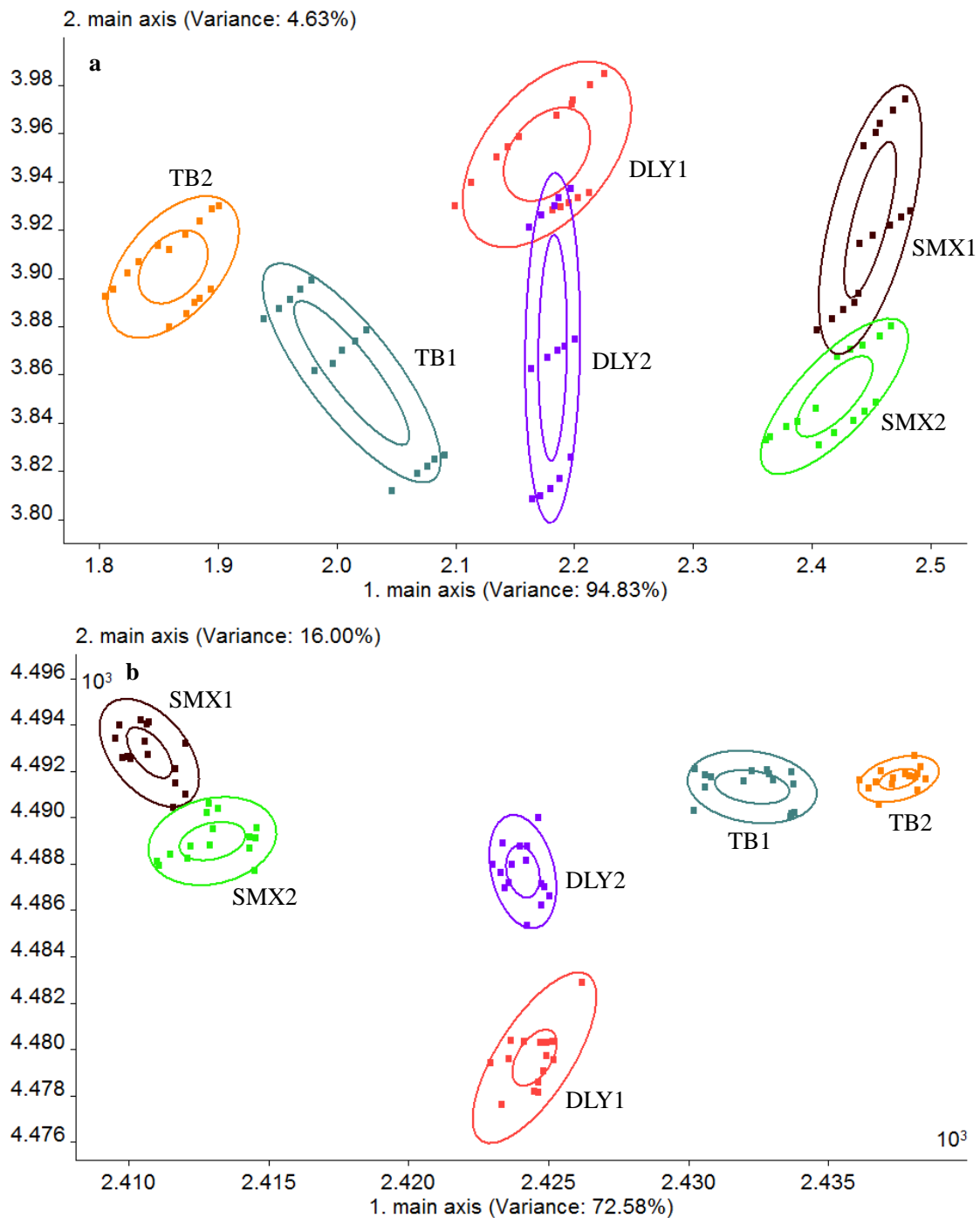


Fig. 4 PCA (a) and LDA (b) plot of e-nose response from different boiled pork (TB1 = *Triceps Brachii* muscle of Tibetan pigs, TB2 = *Biceps Femoris* muscle of Tibetan pigs, DLY1 = *Triceps Brachii* muscle of Duroc × (Landrace × Yorkshire), DLY2 = *Biceps Femoris* muscle of Duroc × (Landrace × Yorkshire), SMX1 = *Triceps Brachii* muscle of Sanmenxia pigs, SMX2 = *Biceps Femoris* muscle of Sanmenxia pigs).

Table 1. Performance description and sensitivity of metal oxide sensors for PEN3 electronic nose

No.	Sensor name	Performance description	Reference
1	W1C	Benzene and aromatic compounds	Methylbenzene, 10 ppm
2	W5S	Broad range sensitivity, very sensitive to nitrogen oxides	Nitrogen dioxide, 1 ppm
3	W3C	Ammonia, sensitive to aromatic compounds	Benzene, 10 ppm
4	W6S	Mainly hydrogen, selectively	Hydrogen, 100 ppm
5	W5C	Alkane, aromatic compounds	Propane, 1 ppm
6	W1S	Sensitive to methane, broad range.	Methane, 100 ppm
7	W1W	Sensitive to many sulfur organic compounds and terpenes.	Hydrogen sulfide, 1 ppm
8	W2S	Alcohol, sensitive to aromatic compounds with broad range, similar to No. 6.	Carbon monoxide, 100 ppm
9	W2W	Aromatic compounds and sulfur organic compounds	Hydrogen sulfide, 1 ppm
10	W3S	Reacts on high concentrations, very sensitive to several compounds	Methane, 100 ppm

Table 2. Concentration ratios and quantities of volatile composition of boiled pork from different breeds of pigs.

Classes of components	Ratio% (quantities)									Breeds and parts DLY-SMX-TB
	<i>Triceps Brachii</i> muscle			<i>Biceps Femoris</i> muscle			<i>Triceps Brachii</i> and <i>Biceps Femoris</i> muscle			
	DLY	SMX	TB	DLY	SMX	TB	DLY	SMX	TB	
Aldehydes	56.5 (16)	50.0 (18)	65.7 (12)	55.1 (20)	56.2 (17)	64.2 (23)	55.1-56.5 (21)	50.0-56.2 (20)	64.2-65.7 (24)	50.5-65.7 (25)
Alcohols	8.6 (5)	9.2 (4)	4.8 (6)	10.3 (6)	8.5 (3)	6.1 (4)	8.6-10.3 (7)	8.5-9.2 (4)	4.8-6.1 (6)	4.8-10.3 (7)
Ketones	0.6 (1)	0.4 (1)	0.5 (1)	0.4 (1)	0.5 (1)	0.9 (1)	0.4-0.6 (1)	0.4-0.5 (1)	0.5-0.9 (1)	0.4-0.9 (1)
Ethers	8.0 (1)	12.3 (3)	3.6 (1)	6.6 (1)	9.0 (2)	2.8 (1)	6.6-8.0 (1)	9.0-12.3 (3)	2.8-3.6 (1)	2.8-12.3 (3)
Aromatics	8.4 (7)	0.6 (2)	3.4 (4)	7.3 (7)	0.9 (3)	4.4 (8)	7.3-8.4 (7)	0.6-0.9 (3)	3.4-4.4 (8)	0.6-8.4 (8)
Hydrocarbons	3.4 (4)	2.4 (4)	2.1 (3)	3.1 (6)	3.8 (3)	5.7 (5)	3.1-3.4 (6)	2.4-3.8 (4)	2.1-5.7 (5)	2.1-5.7 (8)
Furans	5.4 (3)	7.7 (3)	5.3 (3)	4.9 (3)	6.7 (3)	5.8 (3)	4.9-5.4 (3)	6.7-7.7 (3)	5.3-5.8 (3)	5.3-7.7 (3)
N-containing compounds	5.0 (2)	8.0 (2)	6.5 (2)	6.0 (2)	5.8 (2)	3.9 (2)	5.0-6.0 (2)	5.8-8.0 (2)	3.9-6.5 (2)	3.9-6.5 (2)
S-containing compounds	4.0 (4)	9.5 (4)	8.1 (4)	6.2 (4)	8.6 (4)	6.2 (4)	4.0-6.2 (4)	8.6-9.5 (4)	6.2-8.1 (4)	4.0-9.5 (4)
Total	100.0 (43)	100.0 (41)	100.0 (36)	100.0 (50)	100.0 (38)	100.0 (51)	100.0 (52)	100.0 (44)	100.0 (54)	100.0 (61)

Note: DLY, Duroc × (Landrace × Yorkshire); SMX, Sanmenxia pig; TB, Tibetan pig.

Table 3. Identification and quantification of volatile compounds in boiled pork from different breeds of pigs by GC-MS/O ($\mu\text{g}\cdot\text{kg}^{-1}$).

Compounds	¹ DB-Wax	² DB-5	³ Identification	<i>Triceps Brachii</i> muscle					<i>Biceps Femoris</i> muscle					Sign. parts		
				DLY	SMX	TB	SEM	Sign.	DLY	SMX	TB	SEM	Sign.	DLY	SMX	TB
Aldehydes (25)				3506.0 _a ^x	1626.5 _c ^y	2832.6 _b ^y	275.4	***	2158.4 _b ^y	2023.7 _b ^x	3511.9 _a ^x	238.7	***	***	**	***
2,3-Dimethylpentanal	<900	-	MS,RI	0.0 _b ^y	0.0 _b ^y	13.8 _a ^x	2.3	***	2.8 _c ^x	4.7 _b ^x	13.0 _a ^x	1.6	***	***	***	NS
Pentanal	927	702	MS,RI,O	41.7 _b ^y	11.7 _c ^y	145.3 _a ^x	20.2	***	60.9 _a ^x	26.0 _b ^x	0.0 _c ^y	8.8	***	**	***	***
Hexanal	1074	797	MS,RI,O	2620.9 _a ^x	1128.3 _c ^y	1509.5 _b ^y	224.5	***	1373.3 _a ^y	1341.1 _a ^x	1069.6 _b ^x	51.1	**	***	*	**
Heptanal	1175	900	MS,RI,O	125.2 _a ^x	56.9 _c ^x	96.7 _b ^x	10.2	***	85.3 _b ^y	62.0 _c ^x	91.6 _a ^x	4.6	***	**	NS	NS
Octanal	1281	1002	MS,RI,O	82.2 _b ^x	0.0 _c ^y	87.0 _a ^x	14.1	***	57.0 _c ^y	62.2 _b ^x	85.1 _a ^y	4.4	***	***	***	*
Nonanal	1388	1112	MS,RI,O	333.8 _b ^x	255.7 _c ^x	454.6 _a ^x	29.4	***	267.4 _b ^y	248.7 _b ^x	362.6 _a ^y	18.1	***	**	NS	**
(<i>E</i>)-2-Octenal	1424	1062	MS,RI,O	47.2 _a ^x	21.4 _b ^x	23.0 _b ^x	4.2	***	31.3 _a ^y	23.3 _c ^x	27.0 _b ^x	1.3	**	**	NS	NS
(<i>E,E</i>)-2,4-Heptadienal	1486	1015	MS,RI,O	0.0 _b	0.0 _b	9.2 _a ^y	1.5	***	0.0 _b	0.0 _b ^y	10.8 _a ^x	1.8	***	NS	NS	**
Decanal	1494	1210	MS,RI,O	0.0 _b ^y	11.0 _a ^x	0.0 _b ^y	1.8	***	21.7 _a ^x	0.0 _c ^y	13.8 _b ^x	3.2	***	***	***	***
Benzaldehyde	1514	926	MS,RI,O	135.9 _b ^x	60.2 _c ^y	294.5 _a ^x	34.5	***	107.2 _b ^y	113.0 _b ^x	276.3 _a ^y	27.8	***	**	***	*
(<i>E</i>)-2-Nonenal	1531	1165	MS,RI,O	16.6 _a ^x	8.7 _c ^y	13.9 _b ^y	1.2	***	14.2 _b ^y	13.0 _b ^x	18.6 _a ^x	0.9	***	***	**	***
cis-4-Decenal	1534	1202	MS,RI	22.4 _a ^x	19.2 _b ^x	0.0 _c ^y	3.5	***	24.0 _a ^x	13.5 _b ^y	15.2 _b ^x	1.7	***	NS	***	***
(<i>E</i>)-2-Decenal	1600	1265	MS,RI,O	0.0 _b	13.6 _a ^y	0.0 _b ^y	2.3	***	0.0 _c	18.1 _a ^x	14.2 _b ^x	2.8	***	NS	**	***
2-Butyl-2-octenal	1665	1392	MS,RI	13.3 _a	7.0 _b ^x	0.0 _c	1.9	***	20.7 _b	7.2 _c ^x	21.3 _a	2.3	***	***	NS	***
9,12,15-Octadecatrienal	1675	-	MS,RI	0.0 _b	5.3 _a ^x	0.0 _b	0.9	***	0.0	0.0 ^y	0.0	0.0	NS	NS	***	NS
(<i>E,E</i>)-2,4-Nonadienal	1695	1219	MS,RI,O	8.9 _a ^x	4.1 _b ^x	0.0 _c ^y	1.3	***	7.6 _a ^x	4.1 _b ^x	7.4 _a ^x	0.6	**	NS	NS	**
4-Ethylbenzaldehyde	1701	1173	MS,RI	10.8 _b ^x	5.5 _c ^y	33.5 _a ^y	4.3	***	9.1 _b ^x	9.4 _b ^x	69.5 _a ^x	10.0	***	NS	*	***
Dodecanal	1706	1418	MS,RI,O	4.5 _a ^y	2.9 _b ^y	0.0 _c ^y	0.7	***	7.2 _b ^x	6.0 _b ^x	23.6 _a ^x	2.8	***	**	**	***
2-Undecenal	1747	1153	MS,RI,O	7.3 _a ^x	0.0 _b	0.0 _b ^y	1.2	***	0.0 _b ^y	0.0 _b	19.9 _a ^x	3.5	**	***	NS	**
(<i>E,E</i>)-2,4-Decadienal	1758	1324	MS,RI,O	11.4 _a ^x	5.8 _b ^x	0.0 _c ^y	1.7	***	4.0 _b ^y	0.0 _c ^y	7.5 _a ^x	1.1	***	**	**	***
Tridecanal	1812	1525	MS,RI	0.0 _b ^y	3.9 _a ^y	0.0 _b ^y	0.7	***	10.8 _b ^x	12.1 _b ^x	67.0 _a ^x	9.3	***	***	***	***
Tetradecanal	1918	1626	MS,RI	0.0 ^y	0.0	0.0 ^y	0.0	NS	9.4 _b ^x	0.0 _c	85.1 _a ^x	13.5	***	***	NS	***
4-Pentylbenzaldehyde	1999	1472	MS,RI	0.0 ^y	0.0	0.0 ^y	0.0	NS	2.0 _b ^x	0.0 _c	11.3 _a ^x	1.8	***	***	NS	***
Pentadecanal	2025	1712	MS,RI	0.0	0.0	0.0 ^y	0.0	NS	0.0 _b	0.0 _b	211.8 _a ^x	35.3	***	NS	NS	***

Hexadecanal	2132	1832	MS,RI	24.0 _b ^y	5.3 _b ^y	151.5 _a ^y	23.2	***	42.4 _c ^x	59.2 _b ^x	989.6 _a ^x	156.5	***	**	***	***
Alcohols (7)				534.2 _a ^x	298.6 _b ^x	208.9 _c ^y	48.7	***	405.1 _a ^y	305.5 _b ^x	331.8 _b ^x	15.9	**	**	NS	**
1-Pentanol	1245	756	MS,RI,O	72.7 _a ^x	32.0 _b ^x	31.4 _b ^x	6.9	***	37.3 _a ^y	0.0 _b ^y	0.0 _b ^y	6.2	***	***	***	***
1-Hexanol	1347	865	MS,RI,O	20.7 _a ^x	0.0 _c	16.2 _b ^y	3.1	***	0.0 _b ^y	0.0 _b	116.1 _a ^x	19.4	***	***	NS	***
1-Octen-3-ol	1443	981	MS,RI,O	388.6 _a ^x	228.9 _b ^y	113.7 _c ^y	40.0	***	306.4 _a ^y	257.8 _b ^x	155.0 _c ^x	22.9	***	**	*	*
2-Hexyldecanol	1546	-	MS,RI	0.0 ^y	0.0	0.0	0.0	NS	5.3 _a ^x	0.0 _b	0.0 _b	0.9	***	***	NS	NS
1-Octanol	1552	1072	MS,RI,O	19.6 _a ^x	13.3 _b ^y	19.3 _a ^x	1.1	**	20.3 _b ^x	22.5 _a ^x	19.7 _b ^x	0.5	*	NS	***	NS
(E)-2-Octen-1-ol	1608	1079	MS,RI,O	32.7 _a ^x	24.4 _b ^x	13.6 _c ^y	2.8	***	30.6 _a ^x	25.2 _b ^x	19.0 _c ^x	1.8	**	NS	NS	**
Anethole	1818	-	MS,RI,O	0.0 _b ^y	0.0 _b	14.7 _a ^y	2.5	***	5.2 _b ^x	0.0 _c	22.0 _a ^x	3.3	***	***	NS	***
Ketones (1)				35.0 _a ^x	13.6 _c ^x	23.6 _b ^y	3.1	***	16.8 _b ^y	16.3 _b ^x	47.3 _a ^x	5.1	***	***	NS	***
(E,Z)-3,5-Octadien-2-one	1564	1094	MS,RI,O	35.0 _a ^x	13.6 _c ^x	23.6 _b ^y	3.1	***	16.8 _b ^y	16.3 _b ^x	47.3 _a ^x	5.1	***	***	NS	***
Esters (3)				498.7 _a ^x	398.8 _b ^x	156.2 _c ^x	51.3	***	259.2 _b ^y	322.7 _a ^y	155.1 _c ^x	25.0	***	***	**	NS
Terpinyl acetate	1186	1361	MS,RI,O	0.0 _b	18.1 _a ^x	0.0 _b	3.0	***	0.0	0.0 ^y	0.0	0.0	NS	NS	***	NS
Ethyl hexanoate	1225	1006	MS,RI,O	0.0 _b	7.8 _a ^y	0.0 _b	1.3	***	0.0 _b	8.9 _a ^x	0.0 _b	1.5	***	NS	**	NS
Vinyl hexanoate	1313	-	MS,RI	498.7 _a ^x	372.9 _b ^x	156.2 _c ^x	50.3	***	259.2 _b ^y	313.8 _a ^y	155.1 _c ^x	23.8	***	***	**	NS
Aromatics (8)				523.0 _a ^x	19.2 _c ^y	148.0 _b ^y	75.6	***	285.5 _a ^y	34.0 _c ^x	239.5 _b ^x	38.7	***	***	***	***
Ethylbenzene	1112	872	MS,RI,O	47.8 _a ^x	0.0 _b	0.0 _b ^y	8.0	***	24.9 _a ^y	0.0 _c	8.4 _b ^x	3.7	***	***	NS	***
p-Xylene	1119	868	MS,RI	71.8 _a ^x	0.0 _c	34.7 _b ^x	10.4	***	40.5 _a ^y	0.0 _c	35.8 _b ^x	6.4	***	***	NS	NS
o-Xylene	1126	888	MS,RI	96.9 _a ^x	0.0 _b	0.0 _b ^y	16.2	***	56.5 _a ^y	0.0 _c	34.1 _b ^x	8.2	***	***	NS	
Styrene	1246	895	MS,RI,O	244.1 _a ^x	0.0 _c	91.3 _b ^y	35.6	***	131.3 _a ^y	0.0 _c	111.7 _b ^x	20.5	***	***	NS	**
1,2,4-Trimethylbenzene	1271	945	MS,RI	15.9 _a ^x	0.0 _b ^y	0.0 _b ^y	2.7	***	4.5 _b ^y	8.0 _a ^x	4.5 _b ^x	0.6	**	***	***	**
1,2,4,5-Tetramethylbenzene	1418	1218	MS,RI	37.2 _a ^x	15.0 _b ^y	0.0 _c ^y	5.4	***	21.9 _a ^y	20.1 _a ^x	13.2 _b ^x	1.4	**	**	**	***
Naphthalene	1733	-	MS,RI,O	9.2 _b ^x	4.2 _c ^y	13.2 _a ^y	1.3	***	5.9 _b ^y	5.9 _b ^x	18.6 _a ^x	2.1	***	**	*	**
2-Methylnaphthalene	1845	-	MS,RI	0.0 _b	0.0 _b	8.7 _a ^y	1.5	***	0.0 _b	0.0 _b	13.4 _a ^x	2.2	***	NS	NS	***
Hydrocarbons (8)				212.2 _a ^x	76.8 _c ^y	91.5 _b ^y	21.5	***	122.6 _b ^y	135.6 _b ^x	314.2 _a ^x	31.1	***	***	***	***
Limonene	1190	1032	MS,RI,O	0.0 ^y	0.0	0.0	0.0	NS	14.4 _a ^x	0.0 _b	0.0 _b	2.4	***	***	NS	NS
Dodecane	1195	1104	MS,RI,O	39.7 _a ^x	15.7 _b ^x	0.0 _c	5.8	***	10.4 _a ^y	0.0 _b ^y	0.0 _b	1.7	***	***	***	NS
Tridecane	1296	1301	MS,RI	42.7 _a ^x	20.9 _c ^y	24.6 _b ^y	3.4	***	25.3 _b ^y	52.2 _a ^x	43.1 _a ^x	4.2	**	**	**	**
3-Methyltridecane	1368	-	MS,RI	0.0 ^y	0.0	0.0	0.0	NS	6.4 _a ^x	0.0 _b	0.0 _b	1.1	***	***	NS	NS

Tetradecane	1400	1401	MS,RI	103.3 _a ^x	29.0 _c ^y	32.5 _b ^y	12.1	***	44.2 _b ^y	46.8 _b ^x	94.8 _a ^x	8.2	***	***	**	***
Pentadecane	1501	1500	MS,RI	26.5 _b ^x	11.2 _c ^y	34.4 _a ^y	3.4	***	21.8 _c ^y	36.6 _b ^x	138.2 _a ^x	18.4	***	**	***	***
Longifolene	1574	1403	MS,RI	0.0	0.0	0.0 ^y	0.0	NS	0.0 _b	0.0 _b	16.4 _a ^x	2.7	***	NS	NS	***
Hexadecane	1600	1600	MS,RI	0.0	0.0	0.0 ^y	0.0	NS	0.0 _b	0.0 _b	21.6 _a ^x	3.6	***	NS	NS	***
Furans (3)				335.7 _a ^x	251.5 _b ^x	226.6 _c ^y	16.9	***	192.0 _c ^y	240.0 _b ^y	316.3 _a ^x	18.2	***	***	NS	***
2-Ethylfuran	952	708	MS,RI,O	39.8 _b ^x	42.5 _b ^y	52.2 _a ^x	2.0	***	41.1 _c ^x	50.6 _a ^x	47.7 _b ^x	1.4	***	NS	**	NS
2-Pentylfuran	1218	988	MS,RI,O	244.0 _a ^x	162.6 _b ^x	132.6 _c ^y	17.0	***	108.5 _c ^y	155.5 _b ^x	231.4 _a ^x	18.0	***	***	NS	***
2-Furanmethanol	1625	851	MS,RI,O	51.9 _a ^x	46.5 _b ^x	41.8 _c ^x	1.5	***	42.4 _a ^y	33.9 _c ^y	37.3 _b ^y	1.3	**	**	**	*
N-containing compounds (2)				311.0 _a ^x	259.4 _c ^x	278.3 _b ^x	7.6	***	233.5 _a ^y	209.7 _b ^y	214.6 _b ^y	3.7	***	***	***	***
Pyridine	1156	751	MS,RI,O	223.0 _a ^x	175.2 _c ^x	183.6 _b ^x	7.4	***	182.7 _a ^y	157.5 _b ^y	152.8 _c ^y	4.7	***	***	**	***
2-Acetylpyrazine	1978	1095	MS,RI,O	88.0 _b ^x	84.3 _c ^x	94.7 _a ^x	1.5	***	50.8 _b ^y	52.2 _b ^y	61.8 _a ^y	1.8	***	***	***	***
S-containing compounds (4)				246.4 _c ^x	309.1 _b	347.5 _a	14.8	***	243.7 _c ^x	310.4 _b	341.8 _a	14.5	***	NS	NS	NS
Dimethyl disulphide	1109	785	MS,RI,O	84.5 _c ^x	128.2 _b ^x	155.5 _a ^x	10.4	***	54.0 _c ^y	116.5 _b ^y	140.2 _a ^y	12.9	***	**	**	***
3-Methylthiophene	1185	773	MS,RI,O	20.3 _a ^y	17.2 _b ^y	18.2 _{ab} ^y	0.6	***	25.7 _a ^x	27.4 _a ^x	25.4 _a ^x	0.5	NS	**	**	**
2-Acetylthiazole	1632	1016	MS,RI,O	32.8 _b ^y	68.3 _a ^x	69.5 _a ^x	6.0	***	42.6 _b ^x	49.9 _a ^y	51.3 _a ^y	1.4	***	**	***	***
Benzothiazole	1934	1225	MS,RI,O	108.7 _a ^y	95.4 _c ^y	104.3 _b ^y	2.0	***	121.5 _{ab} ^x	116.7 _b ^x	124.9 _a ^x	1.5	*	***	***	**
Total				6202.1 _a ^x	3253.6 _c ^y	4313.2 _b ^y	431.5	***	3916.6 _b ^y	3597.8 _c ^x	5472.4 _a ^x	291.0	***	***	*	***

Note: DLY, Duroc × (Landrace × Yorkshire); SMX, Sanmenxia pig; TB, Tibetan pig. ^{a-b}means in the same row not followed by a common subscript letter differ significantly ($P < 0.05$, Duncan test) (differences among the breeds of pigs). ^{x-y}means in the same row not followed by a common superscript letter differ significantly ($P < 0.05$, Duncan test) (differences between *Triceps Brachii* and *Biceps Femoris* muscle of pigs). All experiments done in n=3 independent boiled pork samples. Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$; NS, not significant; SEM, standard error of the mean.

¹ Linear retention index calculated on DB-Wax capillary column.

² Linear retention index calculated on DB-5 capillary column.

³ Means of identification: MS, mass spectrum comparison using NIST libraries; RI, retention index compared with literature value; O, aroma description (odor).

Table 4. Odor-active compounds (OACs, OAVs > 1) in boiled pork from different breeds of pigs.

Compounds	¹ Odor thresholds ($\mu\text{g}\cdot\text{kg}^{-1}$)	² Odor descriptions	<i>Triceps Brachii</i> muscle					<i>Biceps Femoris</i> muscle					Sign. parts		
			DLY	SMX	TB	SEM	Sign.	DLY	SMX	TB	SEM	Sign.	DLY	SMX	TB
Pentanal	9	Fruity	4.6 _b ^y	1.3 _c ^y	16.1 _a ^x	2.2	***	6.8 _a ^x	2.9 _b ^x	0.0 _c ^y	1.0	***	**	***	***
Hexanal	5	Green, grass	524.2 _a ^x	225.7 _c ^y	301.9 _b ^x	44.9	***	274.7 _a ^y	268.2 _a ^x	213.9 _b ^y	10.2	**	***	*	**
Heptanal	3	Fatty, putty	41.7 _a ^x	19.0 _c ^x	32.2 _b ^x	3.4	***	28.4 _b ^y	20.7 _c ^x	30.5 _a ^x	1.5	***	**	NS	NS
Octanal	0.578	Fatty, pungent	142.2 _b ^x	0.0 _c ^y	150.5 _a ^x	24.4	***	98.5 _c ^y	107.6 _b ^x	147.3 _a ^y	7.5	***	***	***	*
Nonanal	1	Fatty, floral, wax	333.8 _b ^x	255.7 _c ^x	454.6 _a ^x	29.4	***	267.4 _b ^y	248.7 _b ^x	362.6 _a ^y	18.1	***	**	NS	**
(<i>E</i>)-2-Octenal	3	Burdock, fatty	15.7 _a ^x	7.1 _b ^x	7.7 _b ^x	1.4	***	10.4 _a ^y	7.8 _c ^x	9.0 _b ^x	0.4	**	**	NS	NS
Decanal	2	Orange peel, soapy	0.0 _b ^y	5.5 _a ^x	0.0 _b ^y	0.9	***	10.9 _a ^x	0.0 _c ^y	6.9 _b ^x	1.6	***	***	***	***
Benzaldehyde	41.7	Bitter, almond	3.3 _b ^x	1.4 _c ^y	7.1 _a ^x	0.8	***	2.6 _b ^y	2.7 _b ^x	6.6 _a ^y	0.7	***	**	***	*
(<i>E</i>)-2-Nonenal	1	Cardboard, cucumber	16.6 _a ^x	8.7 _c ^y	13.9 _b ^y	1.2	***	14.2 _b ^y	13.0 _b ^x	18.6 _a ^x	0.9	***	***	**	***
(<i>E</i>)-2-Decenal	0.4	Fatty, green	0.0 _b	34.0 _a ^y	0.0 _b ^y	5.7	***	0.0 _c	45.2 _a ^x	35.5 _b ^x	6.9	***	NS	**	***
(<i>E,E</i>)-2,4-Nonadienal	0.16	Fatty, green	55.7 _a ^x	25.7 _b ^x	0.0 _c ^y	8.2	***	47.5 _a ^x	25.3 _b ^x	45.9 _a ^x	4.0	**	NS	NS	**
Dodecanal	2	Herbaceous, fatty	2.2 _a ^y	1.5 _b ^y	0.0 _c ^y	0.3	***	3.6 _b ^x	3.0 _b ^x	11.8 _a ^x	1.4	***	**	**	***
2-Undecenal	0.78	Wax, fatty	9.3 _a ^x	0.0 _b	0.0 _b ^y	1.6	***	0.0 _b ^y	0.0 _b	25.6 _a ^x	4.5	**	***	NS	**
(<i>E,E</i>)-2,4-Decadienal	0.07	Fatty, deep-fried	163.4 _a ^x	83.1 _b ^x	0.0 _c ^y	24.0	***	57.8 _b ^y	0.0 _c ^y	107.0 _a ^x	15.5	***	**	**	***
1-Octen-3-ol	2	Mushroom	194.3 _a ^x	114.5 _b ^x	56.9 _c ^y	20.0	***	153.2 _a ^y	128.9 _b ^x	77.5 _c ^x	11.5	***	**	NS	*
(<i>E</i>)-2-Octen-1-ol	3	Fruity, green apple	10.9 _a ^x	8.1 _b ^x	4.5 _c ^y	0.9	***	10.2 _a ^x	8.4 _b ^x	6.3 _c ^x	0.6	**	NS	NS	**
Anethole	15	Rubber, paint	0.0 _b ^y	0.0 _b	1.0 _a ^y	0.2	***	0.3 _b ^x	0.0 _c	1.5 _a ^x	0.2	***	***	NS	***
Ethyl hexanoate	1	Fatty, green	0.0 _b	7.8 _a ^y	0.0 _b	1.3	***	0.0 _b	8.9 _a ^x	0.0 _b	1.5	***	NS	**	NS
Styrene	65	Herbaceous, fatty	3.8 _a ^x	0.0 _c	1.4 _b ^y	0.5	***	2.0 _a ^y	0.0 _c	1.7 _b ^x	0.3	***	***	NS	**
2-Ethylfuran	2.3	Rubber, pungent	17.3 _b ^x	18.5 _b ^y	22.7 _a ^x	0.8	***	17.9 _c ^x	22.0 _a ^x	20.7 _b ^x	0.6	***	NS	**	NS
2-Pentylfuran	6	Pungent, sweet	40.7 _a ^x	27.1 _b ^x	22.1 _c ^y	2.8	***	18.1 _c ^y	25.9 _b ^x	38.6 _a ^x	3.0	***	***	NS	***
Dimethyl disulphide	1.1	Cooked cabbage	76.8 _c ^x	116.5 _b ^x	141.3 _a ^x	9.4	***	49.1 _c ^y	105.9 _b ^y	127.4 _a ^y	11.7	***	**	**	***
2-Acetylpyrazine	62	Nutty, popcorn	1.4 _b ^x	1.4 _b ^x	1.5 _a ^x	0.02	***	0.8 _b ^y	0.8 _b ^y	1.0 _a ^y	0.03	***	***	***	***

2-Acetylthiazole	10	Caramel, sweaty	3.3 _b ^y	6.8 _a ^x	7.0 _a ^x	0.6	***	4.3 _b ^x	5.0 _a ^y	5.1 _a ^y	0.1	***	**	***	***
Benzothiazole	80	Caramel, cheese	1.4 _a ^y	1.2 _c ^y	1.3 _b ^y	0.03	***	1.5 _b ^x	1.5 _b ^x	1.6 _a ^x	0.02	*	***	***	**
Total			1662.6 _a ^x	970.6 _c ^x	1243.7 _b ^x	100.9	***	1080.0 _b ^y	1052.6 _b ^x	1302.5 _a ^x	40.8	***	***	NS	NS

Note: DLY, Duroc × (Landrace × Yorkshire); SMX, Sanmenxia pig; TB, Tibetan pig. ^{a-b}means in the same row not followed by a common subscript letter differ significantly ($P < 0.05$, Duncan test) (differences among the breeds of pigs). ^{x-y}means in the same row not followed by a common superscript letter differ significantly ($P < 0.05$, Duncan test) (differences between *Triceps Brachii* and *Biceps Femoris* muscle of pigs). All experiments done in n=3 independent boiled pork samples. Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$; NS, not significant; SEM, standard error of the mean.

¹ Odor thresholds were mainly obtained from the literature and an online database, with water applied as the matrix: (Gu, S. Q., Wang, X. C., Tao, N. P., & Wu, N, 2013; Sansone-Land, A., Takeoka, G.R., & Shoemaker, C. F, 2014; Czerny et al., 2008; Mayuoni-Kirshinbaum, L., Daus, A., & Porat, R, 2013; Liu, Y., He, C., & Song, H, 2018), (<http://www.flavornet.org>, <http://www.odour.org.uk>).

² Odor descriptions were mainly gathered from the following literature and online database: (Gu, S. Q., Wang, X. C., Tao, N. P., & Wu, N, 2013; Czerny et al. 2008; Mayuoni-Kirshinbaum, L., Daus, A., & Porat, R, 2013), (<http://www.flavornet.org>).

Table 5. Information of PCA, PLS-DA and one way analysis of variance.

Odor-active compounds	PCA		PLS-DA	One way analysis of variance
	PC1	PC2	VIP score	<i>p</i> value
Pentanal	-0.236	0.468	0.894	< 0.01
Hexanal	0.727	0.041	1.026	< 0.01
Heptanal	0.740	0.541	1.028	< 0.01
Octanal	0.386	0.791	0.947	< 0.01
Nonanal	-0.061	0.923	0.852	< 0.01
(<i>E</i>)-2-Octenal	0.945	0.006	1.069	< 0.01
Decanal	0.119	-0.225	0.750	< 0.01
Benzaldehyde	-0.113	0.988	0.883	< 0.01
(<i>E</i>)-2-Nonenal	0.617	0.671	1.011	< 0.01
(<i>E</i>)-2-Decenal	-0.452	-0.273	0.945	< 0.01
(<i>E,E</i>)-2,4-Nonadienal	0.864	-0.247	1.044	< 0.01
Dodecanal	0.249	0.337	0.871	< 0.01
2-Undecenal	0.387	0.517	0.910	< 0.01
(<i>E,E</i>)-2,4-Decadienal	0.790	-0.046	1.033	< 0.01
1-Octen-3-ol	0.747	-0.598	1.039	< 0.01
(<i>E</i>)-2-Octen-1-ol	0.708	-0.682	1.023	< 0.01
Anethole	-0.129	0.863	0.882	< 0.01
Ethyl hexanoate	-0.555	-0.676	1.002	< 0.01
Styrene	0.892	0.345	1.079	< 0.01
2-Ethylfuran	-0.677	0.527	0.981	< 0.01
2-Pentylfuran,	0.562	0.258	0.973	< 0.01
Dimethyl disulphide	-0.671	0.543	1.040	< 0.01
2-Acetylpyrazine	-0.067	0.309	0.973	< 0.01
2-Acetylthiazole	-0.909	0.158	1.067	< 0.01
Benzothiazole	0.362	0.250	0.897	< 0.01

Note: VIP, variable importance in projection.

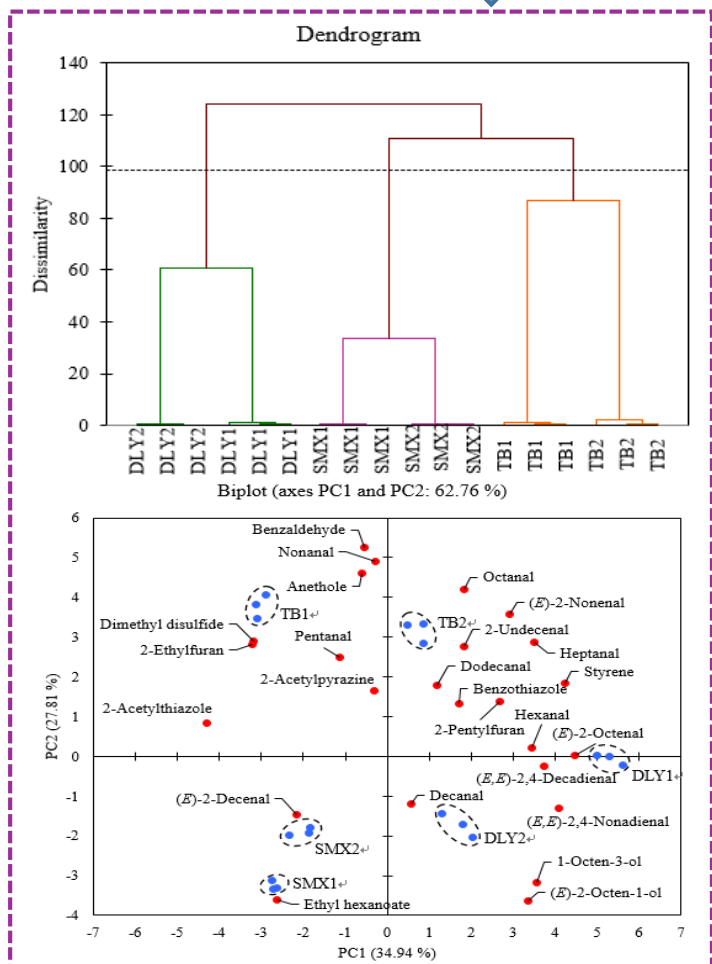
Boiled pork of *Triceps brachii* and *Biceps femoris* muscle from Duroc × (Landrac × Yorkshire)

Boiled pork of *Triceps brachii* and *Biceps femoris* muscle from Sanmenxia pigs

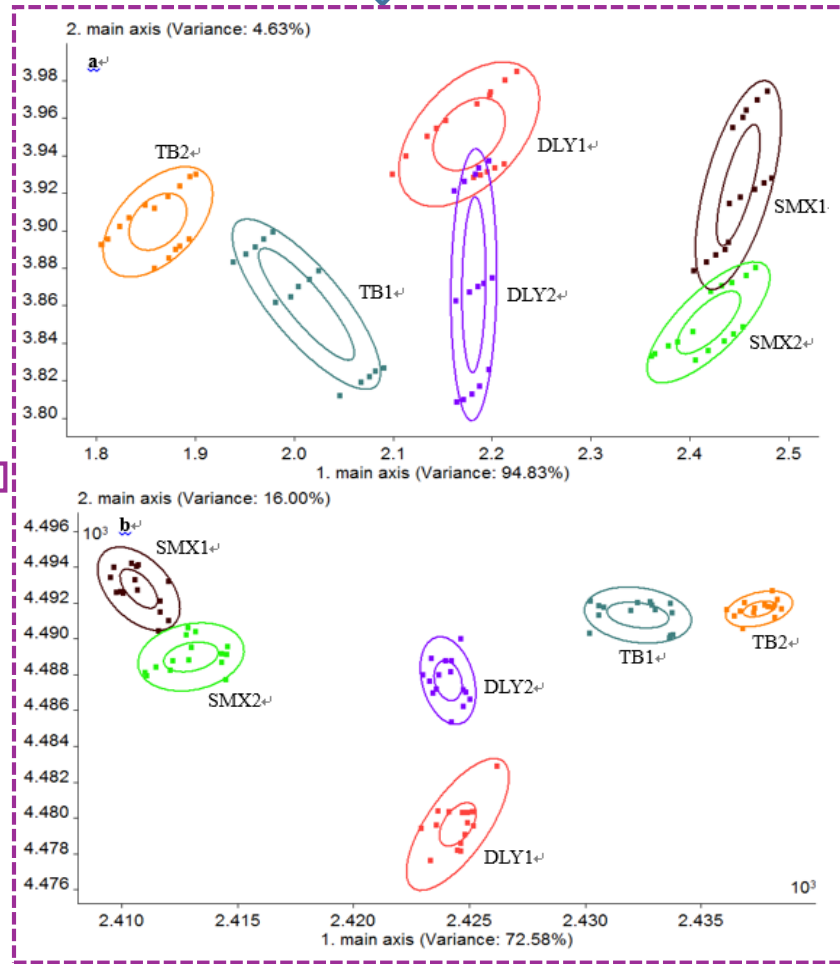
Boiled pork of *Triceps brachii* and *Biceps femoris* muscle from Tibetan pigs

GC-MS/O PCA, AHC & PLS-DA

E-nose PCA & LDA



Characterization and discrimination of boiled pork by volatile compound profiling



Conflicts of Interest:

Dong Han declares that he has no conflict of interest. Chun-Hui Zhang declares that he has no conflict of interest. Marie-Laure Fauconnier declares that she has no conflict of interest. Si Mi declares that she has no conflict of interest.

Author Contributions Section:

D. Han performed the experiments and analysed the results. C-H Zhang contributed to the conception of the research and invaluable experimental design. M-L. Fauconnier and S. Mi critically revised the manuscript.