

**Study on flavor composition and factors influencing the formation of volatile compounds in stewed pork**



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**Résumé**

**Dong Han. (2020). "Étude du profil aromatique et des facteurs influençant la formation des composés volatils dans le ragoût de porc" (Thèse de doctorat en anglais).** Gembloux, Belgique, Gembloux Agro-Bio Tech, Université de Liège, 163 pages, 23 tableaux, 18 figures.

**Résumé:**

Le ragoût de porc traditionnel est un produit mariné en sauce célèbre en Chine obtenu en faisant mijoter le porc frais dans une saumure. La saumure comprenait, dans le cas présent, de l'eau et divers ingrédients tels que du sel, du sucre, des épices, de la sauce de soja et du vin de cuisine. En raison de ses caractéristiques sensorielles uniques, par exemple sa texture tendre, sa couleur vive et sa riche saveur, le ragoût de porc est apprécié des consommateurs. Toutefois, le ragoût de porc traditionnel a également été confronté à un certain nombre de problèmes potentiels ces dernières années. Parmi ceux-ci, le plus important est la volatilisation et la perte de composés aromatiques. Afin de résoudre ce problème, les composés aromatiques de la daube de porc ont fait l'objet d'une analyse complète et une nouvelle technologie de traitement a été proposée.

Dans un premier temps, le profil des composés aromatiques et les composés odorants actifs dans la viande de porc cuite de quatre marques locales, Dahongmen (DHM), Daoxiangcun (DXC), Henghuitong (HHT) et Tianfuhao (TFH), ont été évalués par GC-MS/O et nez électronique combinés à une analyse chimiométrique. Au total, 62 composés volatils ont été identifiés et quantifiés dans tous les échantillons de viande de porc, et 24 d'entre eux ont été considérés comme des composés odorants actifs parce que leur OAV (odor active value, valeur d’activité des odeurs) était supérieure à 1. Selon l'analyse en composants principaux (PCA) et l'analyse partielle des moindres carrés-discriminants (PLS-DA) des données de GC-MS/O et nez électronique, les échantillons de ragoût de porc ont été divisés en trois groupes (DHM, HHT, et DXC-TFH). Le résultat de l'analyse PLS-DA a montré que 9 composés odorants (heptanal, nonanal, delta 3-carène, D-limonène, β-phellandrene, p-cymène, eugénol, 2-éthylfurane et 2-pentylfurane) ont été confirmés comme marqueurs potentiels de l'arôme pour la discrimination des ragoûts de porc.

Ensuite, l’influence des différentes races de porc sur les profils aromatiques de la viande de porc bouillie ont été étudiés. Les trois variétés de porcs sont les suivantes : Tibetan, Sanmenxia et Duroc × (Landrace × Yorkshire). Les composés volatils et les composés odorants actifs dans la viande de porc bouillie provenant de trois races de porcs différentes ont été identifiés et quantifiés par chromatographie en phase gazeuse, olfactométrie, spectrométrie de masse (GC-MS/O) et grâce à la valeur d'activité des odeurs (OAV). Au total, 61 composés volatils ont été identifiés, parmi lesquels 25 composés ont été sélectionnés comme composés odorants actifs dans la viande de porc bouillie. L'hexanal, le nonanal, le 1-octen-3-ol, le disulfure de diméthyle, l'heptanal, le 2-pentylfurane et le 2-éthylfurane sont les principaux responsables de la saveur du porc bouilli. Le profil de saveur a été déterminé par nez électronique sur la base de l'analyse en composants principaux (PCA). Ce résultat a montré que la viande de porc bouillie des trois races de porcs pouvait être clairement distinguée. Le graphique de la valeur de réponse des capteurs du nez électronique a montré que le porc bouilli de différentes races de porcs avait une saveur significativement différente mais, que le porc bouilli des muscles de la patte avant et de la patte arrière des porcs avait des compositions d'arômes similaires. Les analyses ci-dessus ont montré que les différentes races de porcs avaient une plus grande influence sur la saveur du porc bouilli que les morceaux différents de porc, et que la GC-MS/O combinée avec le nez électronique était une méthode adaptée pour déterminer et distinguer les profils de composés volatils des différents échantillons de porc.

En outre, l'influence des différentes recettes d'assaisonnement (SP1 : ragoût de porc à l'eau, SP2 : ragoût de porc à l'eau et au sel, SP3 : ragoût de porc à l'eau, au sel et aux épices, SP4 : ragoût de porc à l'eau, au sel, aux épices et à la sauce soja, SP5 : ragoût de porc à l'eau, au sel, aux épices, à la sauce soja et au sucre, SP6 : ragoût de porc à l'eau, au sel, aux épices, à la sauce soja, au sucre et au vin de cuisine) sur les profils volatils et l'évaluation sensorielle du ragoût de porc ont été étudiés. La GC-MS/O et la chromatographie gazeuse bidimensionnelle combinée à la spectrométrie de masse à temps de vol (GC × GC-TOFMS) ont été appliquées pour détecter le profil de saveur dans le ragoût de porc préparé selon les différentes recettes précitées. Le ragoût de porc traité avec SP1 et SP2 contenait les composés volatils les plus abondants, en particulier des aldéhydes ce qui indique que le porc cuit avec de l'eau et du sel favorise l'oxydation des lipides et la dégradation des acides aminés. En ACP, les échantillons SP3, SP4, SP5 et SP6 étaient proches les uns des autres pour PC1-PC2, alors que l'échantillon SP3 était situé du côté opposé des échantillons SP4, SP5 et SP6 pour PC1-PC3. Il a été démontré que l'ajout d'épices avait une influence significative sur la saveur du ragoût de porc. L'évaluation sensorielle a révélé que les échantillons SP3, SP4, SP5 et SP6 présentaient une odeur d'épices, une odeur de caramel et une odeur de sauce soja plus prononcées. Ces résultats étaient cohérents avec les résultats de la régression partielle par les moindres carrés (PLSR).

Enfin, les composés volatils et non volatils des ragoûts de porc préparé selon différentes méthodes de transformation (TS : ragoût traditionnel, EST : ragoût traditionnel avec dégradation enzymatique, EST : ragoût traditionnel avec dégradation enzymatique et réaction de Maillard, HS : ragoût préparé à haute température, HSE : ragoût préparé à haute température avec dégradation enzymatique, HSEM : ragoût préparé à haute température avec dégradation enzymatique et réaction de Maillard) ont été analysés. Le ragoût de porc préparé à haute température (HS, HSE et HSEM) avait une teneur plus élevée en composés volatils que le ragoût de porc traditionnel (TS, TSE et TSEM), en particulier l'échantillon HSEM. Les échantillons de ragoût de porc traditionnel et de ragoût de porc préparés à haute température se distinguent clairement par la méthode du nez électronique. Les teneurs en acides aminés de type umami (UAA), en acides aminés doux (SAA) et en acides aminés amers (BAA) du ragoût de porc préparé à haute température étaient significativement plus élevées (P < 0,05) que celles du ragoût de porc traditionnel, dont les teneurs en Asp et en Glu liées au goût umami étaient les plus élevées dans les échantillons HS et HSEM. Le ragoût de porc préparé à haute température présentait des teneurs en nucléotides 5' et en acides gras (FA) inférieures à celles du ragoût de porc traditionnel. Il a été conclu que le ragoût de porc préparé à haute température (HS, HSE et HSEM) pouvait être utilisé comme une méthode efficace pour améliorer le goût et l'odeur du ragoût de porc, de plus, l'échantillon HSEM présentait le profil aromatique le plus intéressant.

**Mots-clés:** GC-MS/O, OAV, ragoût de porc, PCA, nez électronique, composés volatils, composés non volatils, PLSR, marqueurs potentiels de goût

**Abstract**

**Dong Han. (2020). “Study on flavor composition and factors influencing the formation of volatile compounds in stewed pork” (PhD Dissertation in English).** Gembloux, Belgique, Gembloux Agro-Bio Tech, Université de Liège, 163 pages, 23 tables, 18 figures.

**Summary:**

The traditional stewed pork is a famous sauce pickled product in China and often produced by stewing the fresh pork in aged brine. Here, the aged brine included water and various ingredients such as salt, sugar, spices, soy sauce and cooking wine. Because of its unique sensory characteristics, for example tender texture, bright colour and rich flavour, the stewed pork is appreciated by consumers. However, the traditional stewed pork also has faced a number of potential problems in recent years. Among them, the most important is the volatilization and loss of flavour compounds. In order to solve this problem, the flavour compounds in the stewed pork were fully analysed and a new processing technology were proposed.

Firstly, the flavour compounds profile and odour-active compounds in the stewed pork from four local brands, Dahongmen (DHM), Daoxiangcun (DXC), Henghuitong (HHT) and Tianfuhao (TFH), were evaluated by GC-MS/O and E-nose combined with chemometrics analysis. A total of 62 volatile compounds were identified and quantified in all pork samples, and 24 of them were considered as odour-active compounds because their OAVs were greater than 1. According to principal components analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) of GC-MS/O and E-nose data, the stewed pork samples were divided into three groups (DHM, HHT, and DXC-TFH). The PLS-DA result showed that 9 odour-active compounds (heptanal, nonanal, 3-carene, d-limonene, β-phellandrene, *p*-cymene, eugenol, 2-ethylfuran and 2-pentylfuran) were confirmed to the potential flavour markers for the discrimination of stewed pork products.

Secondly, the effects of different pork breeds on the aroma profiles of boiled pork were investigated. The three varieties of pigs are Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire). The volatile compounds and odour-active compounds in boiled pork from three different breeds of pigs were identified and quantified using gas chromatography-olfactometry-mass spectrometry (GC-MS/O) and odour activity values (OAVs). A total of 61 volatile compounds were identified, among which 25 compounds were selected as odour-active compounds in boiled pork. Hexanal, nonanal, 1-octen-3-ol, dimethyl disulphide, heptanal, 2-pentylfuran and 2-ethylfuran were the important contributors to the whole flavour of boiled pork. The flavour profile was determined by E-nose based on principal component analysis (PCA). This result showed that boiled pork from the three pig breeds could be clearly distinguished. The radar chart of response value of E-noes sensors displayed the boiled pork from different pig breeds had significantly different flavour, and that boiled pork from the fore leg and hind leg muscles of pigs had similar aroma compositions. The above analyses showed the different pig breeds had a greater influence on flavour of boiled pork than pigs in different parts, and the GC-MS/O combined with E-nose was a feasible method to determine and distinguish the volatile profiles of different varieties of pork samples.

Besides, the influence of different seasoning recipes (SP1: stewing pork in water, SP2: stewing pork with water and salt, SP3: stewing pork with water, salt and spices, SP4: stewing pork with water, salt, spices and soy sauce, SP5: stewing pork with water, salt, spices, soy sauce and sugar, SP6: stewing pork with water, salt, spices, soy sauce, sugar and cooking wine) on volatile profiles and sensory evaluation of stewed pork were studied. The GC-MS/O and two-dimensional gas chromatography combined with time-of-flight mass spectrometry (GC × GC-TOFMS) were applied to detect the flavour profile in stewed pork form different seasoning recipes. The stewed pork processed using SP1 and SP2 had the most abundant volatile compounds, especially aldehydes. Which indicated that the cooked pork with water and salt promoted lipid oxidation and amino acid degradation. Samples SP3, SP4, SP5 and SP6 were close each other in PC1-PC2, whereas samples SP3 was located on the opposite side of samples SP4, SP5 and SP6 in PC1-PC3 using PCA. It was showed that the addition of spices had a significant influence on the flavour of stewed pork. Sensory evaluation revealed the stronger spicy odour, caramel odour and soy sauce odour were presented in samples SP3, SP4, SP5 and SP6. These results were consistent with the results of partial least squares regression (PLSR).

Finally, the volatile compounds and non-volatile compounds of stewed pork with different processing methods (TS: traditional stewing, TSE: traditional stewing with enzymatic degradation, TSE: traditional stewing with enzymatic degradation and Maillard reaction, HS: high-temperature stewing, HSE: high-temperature stewing with enzymatic degradation, HSEM: high-temperature stewing with enzymatic degradation and Maillard reaction) were analysed. The high-temperature stewed pork (HS, HSE and HSEM) had a higher content of volatile composition than traditional stewed pork (TS, TSE and TSEM), especially sample HSEM. The traditional and high-temperature stewed pork samples clearly distinguished by E-nose method. The contents of umami amino acids (UAAs), sweet amino acids (SAAs) and bitter amino acids (BAAs) of high-temperature stewed pork were higher significantly (*P < 0.05*) than those of traditional stewed pork, of which the content of Asp and Glu related to umami taste were the most in sample HS and HSEM. The high-temperature stewed pork showed the lower contents of 5’-nucleotides and fatty acids (FAs) than traditional stewed pork. It was concluded that the pork with high-temperature stewing (HS, HSE and HSEM) could be used as an effective method to improve the taste and odour of stewed pork, moreover, sample HSEM had a great advantage in the formation of odour compounds.

**Keywords:** GC-MS/O, OAVs, stewed pork, PCA, E-nose, volatile compounds, non-volatile compounds, PLSR, potential flavour markers

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**Tables of Contents**

[**Résumé I**](#_Toc44494984)

[**Abstract III**](#_Toc44494985)

[**Acknowledgments V**](#_Toc44494986)

[**Tables of Contents VI**](#_Toc44494987)

[**List of Figures X**](#_Toc44494988)

[**List of Tables XII**](#_Toc44494989)

[**List of Abbreviations XIII**](#_Toc44494990)

[**Chapter Ⅰ. General Introduction 1**](#_Toc44494992)

[**1. Context 3**](#_Toc44494993)

[**2. Objective 3**](#_Toc44494994)

[**3. Research strategy 4**](#_Toc44494995)

[**4. Literature review 4**](#_Toc44494996)

[***4.1. Overview of stewed meat products* 4**](#_Toc44494997)

[***4.2. Formation paths of flavour compounds* 4**](#_Toc44494998)

[**4.2.1. Thermal degradation reaction 4**](#_Toc44494999)

[**4.2.2. Lipid oxidation 5**](#_Toc44495000)

[**4.2.3. Maillard reaction 5**](#_Toc44495001)

[**4.2.4. Lipid-Maillard interactions 6**](#_Toc44495002)

[***4.3. Factors influencing formation of flavour compounds* 7**](#_Toc44495003)

[**4.3.1. Breeds of meat 7**](#_Toc44495004)

[**4.3.2. Spices 7**](#_Toc44495005)

[**4.3.3. Cooking method 7**](#_Toc44495006)

[***4.4. Extraction methods of flavour compounds* 8**](#_Toc44495007)

[**4.4.1. Headspace extraction 8**](#_Toc44495008)

[**4.4.2. Adsorption extraction 8**](#_Toc44495009)

[**4.4.3. Simultaneous distillation and extraction (SDE) 9**](#_Toc44495010)

[***4.5. Analysis of flavour compounds* 10**](#_Toc44495011)

[**4.5.1. Gas chromatography-olfactometry-mass spectrometry (GC-MS/O) 10**](#_Toc44495012)

[**4.5.2. Two-dimensional gas chromatographic combined with time-of-fight mass spectrometry (GC × GC-TOFMS) 10**](#_Toc44495013)

[**4.5.3. Electronic nose (E-nose) and electronic tongue (E-tongue) 11**](#_Toc44495014)

[**4.5.4. Chemometrics 11**](#_Toc44495015)

[***4.6. Conclusions* 12**](#_Toc44495016)

[**5. Reference 12**](#_Toc44495017)

[**Chapter Ⅱ. Characterization of volatile compounds in Chinese stewed pork by solid phase microextraction gas chromatography-mass spectrometry/olfactometry, electronic nose and chemometrics 19**](#_Toc44495019)

[**1. Introduction 22**](#_Toc44495020)

[**2. Materials and Methods 23**](#_Toc44495021)

[***2.1. Materials and chemicals* 23**](#_Toc44495022)

[***2.2. Solid phase microextraction of volatile compounds* 24**](#_Toc44495023)

[***2.3. GC-MS/O analysis of volatile compounds* 24**](#_Toc44495024)

[***2.4. Identification and quantification of volatile compounds* 24**](#_Toc44495025)

[***2.5. E-nose analysis of stewed pork* 25**](#_Toc44495026)

[***2.6. Sensory evaluation of stewed pork* 25**](#_Toc44495027)

[***2.7. Statistical analysis* 26**](#_Toc44495028)

[**3. Results and Discussion 26**](#_Toc44495029)

[***3.1. Volatiles profile of stewed pork characterized by GC-MS/O* 26**](#_Toc44495030)

[**3.1.1. Volatile composition of stewed pork 26**](#_Toc44495031)

[**3.1.2. OAVs of the odour-active compounds 32**](#_Toc44495032)

[***3.2. Discrimination of stewed pork by GC-MS/O* 33**](#_Toc44495033)

[***3.3. Volatile profile of stewed pork characterized by E-nose* 36**](#_Toc44495034)

[***3.4. Discrimination of stewed pork by E-nose* 39**](#_Toc44495035)

[***3.5. Sensory analysis of stewed pork* 40**](#_Toc44495036)

[**4. Conclusions 41**](#_Toc44495037)

[**5. References 41**](#_Toc44495038)

[**Chapter Ⅲ. Characterization and differentiation of boiled pork from Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire) pigs by volatiles profiling and chemometrics analysis 45**](#_Toc44495040)

[**1. Introduction 48**](#_Toc44495041)

[**2. Materials and methods 50**](#_Toc44495042)

[***2.1. Materials and chemicals* 50**](#_Toc44495043)

[***2.2. Boiled pork muscles pre-treatment* 50**](#_Toc44495044)

[***2.3. Solid-phase micro-extraction (SPME) of volatile compounds* 50**](#_Toc44495045)

[***2.4. GC-MS/O analysis* 50**](#_Toc44495046)

[***2.5. Identification and quantification of volatile compounds* 51**](#_Toc44495047)

[***2.6. E-nose analysis* 51**](#_Toc44495048)

[***2.7. Statistical analysis* 52**](#_Toc44495049)

[**3. Results and discussion 52**](#_Toc44495050)

[***3.1. Volatile profiling of boiled pork by GC-MS/O* 52**](#_Toc44495051)

[**3.1.1. Volatile composition of boiled pork 52**](#_Toc44495052)

[**3.1.2. Odour-active compounds in boiled pork 60**](#_Toc44495053)

[***3.2. Discrimination of boiled pork by PCA, PLS-DA and AHC* 63**](#_Toc44495054)

[***3.3. Volatile profiling of boiled pork using E-nose* 66**](#_Toc44495055)

[***3.3. Discrimination of boiled pork by PCA and LDA* 69**](#_Toc44495056)

[**4. Conclusions 71**](#_Toc44495057)

[**5. References 71**](#_Toc44495058)

[**Chapter Ⅳ. Effect of seasoning addition on volatile composition and sensory properties of stewed pork 75**](#_Toc44495060)

[**1. Introduction 78**](#_Toc44495061)

[**2. Materials and methods 79**](#_Toc44495062)

[***2.1. Sampling and chemicals* 79**](#_Toc44495063)

[***2.2. Preparation of stewed pork samples* 80**](#_Toc44495064)

[**2.2.1. Preparation of spice bag 80**](#_Toc44495065)

[**2.2.2. Seasoning formulations and stewing 80**](#_Toc44495066)

[***2.3. Extraction of volatile compounds* 81**](#_Toc44495067)

[***2.4. GC-MS/O analysis* 82**](#_Toc44495068)

[***2.5. GC × GC-TOFMS analysis* 82**](#_Toc44495069)

[***2.6. Quantification of volatile compounds* 82**](#_Toc44495070)

[***2.7. Sensory evaluation* 83**](#_Toc44495071)

[***2.8. Statistical analysis* 84**](#_Toc44495072)

[**3. Results and discussion 84**](#_Toc44495073)

[***3.1. Analysis of volatile components by GC-MS/O and GC × GC-TOFMS* 84**](#_Toc44495074)

[***3.2. Volatile compounds profiling in the fresh and stewed pork* 93**](#_Toc44495075)

[***3.3. Odour-active compounds analysis of the fresh and stewed pork* 103**](#_Toc44495076)

[***3.4. PCA and PLS-DA analysis of odour-active compounds* 107**](#_Toc44495077)

[***3.5. Descriptive sensory analysis* 111**](#_Toc44495078)

[***3.6. Relationship between sensory evaluation and odour-active compounds* 112**](#_Toc44495079)

[**4. Conclusions 116**](#_Toc44495080)

[**5. References 116**](#_Toc44495081)

[**Chapter Ⅴ. Study on the flavour compounds of stewed pork with different processing methods 121**](#_Toc44495083)

[**1. Introduction 124**](#_Toc44495084)

[**2. Materials and methods 125**](#_Toc44495085)

[***2.1. Materials and chemicals* 125**](#_Toc44495086)

[***2.2. Stewed pork with different processing methods* 125**](#_Toc44495087)

[**2.2.1. Traditional stewing with enzymatic degradation and Maillard reaction (TSEM) 125**](#_Toc44495088)

[**2.2.2. High-temperature stewing with enzymatic degradation and Maillard reaction (HSEM) 125**](#_Toc44495089)

[***2.3. Volatile compounds of different stewed pork* 127**](#_Toc44495090)

[**2.3.1. Volatile compounds analysis by GC/MS-O 127**](#_Toc44495091)

[**2.3.2. Electronic nose analysis 128**](#_Toc44495092)

[***2.4. Taste compounds of different stewed pork* 129**](#_Toc44495093)

[**2.4.1. Determination of free amino acids 129**](#_Toc44495094)

[**2.4.2. Determination of 5’-nucleotide analysis 129**](#_Toc44495095)

[**2.4.3. Calculation of equivalent umami concentration (EUC) 130**](#_Toc44495096)

[**2.4.4. Determination of fatty acid 130**](#_Toc44495097)

[**2.4.5. Electronic tongue analysis (E-tongue) 130**](#_Toc44495098)

[***2.5. Statistical analysis* 130**](#_Toc44495099)

[**3. Results and discussion 130**](#_Toc44495100)

[***3.1. Volatile compounds profiling of stewed pork with different processing methods* 130**](#_Toc44495101)

[**3.1.1. Volatile components analysis of stewed pork by GC-MS/O 130**](#_Toc44495102)

[**3.1.2. Odour-active compounds of stewed pork 131**](#_Toc44495103)

[**3.1.3. Volatile composition analysis of stewed pork using E-nose 135**](#_Toc44495104)

[***3.2. Taste compounds profiling of stewed pork with different processing methods* 137**](#_Toc44495105)

[**3.2.1. FAA analysis of stewed pork 137**](#_Toc44495106)

[**3.2.2. 5’-Nucleotide and EUC analysis of stewed pork 138**](#_Toc44495107)

[**3.2.3. Fatty acid composition of stewed pork 139**](#_Toc44495108)

[**3.2.4. Taste composition analysis of stewed pork by E-tongue 140**](#_Toc44495109)

[**4. Conclusions 141**](#_Toc44495110)

[**5. References 142**](#_Toc44495111)

[**Chapter Ⅵ. General discussion, conclusions and perspective 145**](#_Toc44495113)

[**1. General discussion 147**](#_Toc44495114)

[***1.1. Analysis of number of experiment samples* 147**](#_Toc44495115)

[***1.2. Types of volatile compounds and odour-active compounds* 147**](#_Toc44495116)

[***1.3. Analysis methods of volatile compounds in stewed pork* 150**](#_Toc44495117)

[***1.4. Relationship between flavour precursors and volatile compounds* 151**](#_Toc44495118)

[***1.5. Connection between the chapters of flavour research* 151**](#_Toc44495119)

[**2. General conclusion 152**](#_Toc44495120)

[***2.1. Characterization of volatile compounds in Chinese stewed pork using SPME-GC-MS/O and E-nose* 152**](#_Toc44495121)

[***2.2. Characterization and discrimination of boiled pork from different breeds by volatiles profiling and chemomertrics analysis* 152**](#_Toc44495122)

[***2.3. Effect of seasoning addition on volatile composition and sensory properties of stewed pork* 153**](#_Toc44495123)

[***2.4. Determination of volatile and non-volatile compounds of stewed pork from different processing methods* 153**](#_Toc44495124)

[**3. Perspective 153**](#_Toc44495125)

[**4. References 157**](#_Toc44495126)

[**Appendix - publications 163**](#_Toc44495127)

**List of Figures**

[**Figure 1 - 1:** The mechanism of flavour formation through the lipid oxidation. 5](#_Toc44494536)

[**Figure 2 - 1:** (**A**) PCA for odour-active compounds of the four stewed pork. The blue dots represent the samples from stewed pork, and the red dots represent odour-active compounds. (**B**) PLS-DA score plot from different marinated samples (R2X = 0.978, R2Y = 0.997, Q2 = 0.994). The red dots represent DHM, the yellow dots represent HHT, the blue dots represent TFH, and the green dots represent DXC……………………………………………………….35](#_Toc44494537)

[**Figure 2 - 2:** Response curves of E-nose sensors (S1–S10) to DHM, DXC, HHT and TFH. 38](#_Toc44494538)

[**Figure 2 - 3: (A)** Biplot (score plots and load plots) for PCA based on sensor response data. The blue dots represent the samples from stewed pork, and the red dots represent different sensors. (**B**) PLS-DA of E-nose response data for different marinated samples (R2X = 0.997, R2Y = 0.829, Q2 = 0.407). The blue dots represent DHM, the red dots represent HHT, the purple dots represent TFH, and the green dots represent DXC. 40](#_Toc44494539)

[**Figure 2 - 4:** Radar charts of sensory analysis of the stewed pork. 41](#_Toc44494540)

[**Figure 3 - 1:** PCA for odour-active compounds of the different boiled pork (TB1 = fore leg muscle of Tibetan pigs, TB2 = hind leg muscle of Tibetan pigs, DLY1 = fore legmuscle of Duroc × (Landrace × Yorkshire), DLY2 = hind leg muscle of Duroc × (Landrace × Yorkshire), SMX1 = fore legmuscle of Sanmenxia pigs, SMX2 = hind leg muscle of Sanmenxia pigs). The blue dots represent the samples from the different boiled pork, and the red dots respsent odour-active compounds…………………………………………………..………………………………………..64](#_Toc44494541)

[**Figure 3 - 2:** AHC results of the different boiled pork (TB1 = fore leg muscle of Tibetan pigs, TB2 = hind leg muscle of Tibetan pigs, DLY1 = fore legmuscle of Duroc × (Landrace × Yorkshire), DLY2 = hind leg muscle of Duroc × (Landrace × Yorkshire), SMX1 = fore legmuscle of Sanmenxia pigs, SMX2 = hind leg muscle of Sanmenxia pigs). 66](#_Toc44494542)

[**Figure 3 - 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 = fore leg muscle of Tibetan pigs, TB2 = hind leg muscle of Tibetan pigs, DLY1 = fore legmuscle of Duroc × (Landrace × Yorkshire), DLY2 =hind leg muscle of Duroc × (Landrace × Yorkshire), SMX1 = fore legmuscle of Sanmenxia pigs, SMX2 = hind leg muscle of Sanmenxia pigs). 69](#_Toc44494543)

[**Figure 3 - 4:** PCA (a) and LDA (b) plot of e-nose response from different boiled pork (TB1 = fore leg muscle of Tibetan pigs, TB2 = hind leg muscle of Tibetan pigs, DLY1 = fore legmuscle of Duroc × (Landrace × Yorkshire), DLY2 = hind leg muscle of Duroc × (Landrace × Yorkshire), SMX1 = fore legmuscle of Sanmenxia pigs, SMX2 = hind leg muscle of Sanmenxia pigs). 70](#_Toc44494544)

[**Figure 4 - 1:** Flow diagram of the stewed pork. The processing procedures consist of three steps including boiling for 10 min, stewing for 45 min and soaked for 60 min. The sampling points were chosen according to different stewing recipes………………………………………………………………………………..……………………………..81](#_Toc44494545)

[**Figure 4 - 2:** Concentration of volatile compounds of the fresh and stewed pork. Different letters are significantly different (P < 0.05) in each pork treatment group. FP, fresh pork; SP1, boiled pork in water; SP2, cooked pork in water and salt; SP3, stewed pork in water, salt and spices; SP4, stewed pork in water, salt, spices and soy sauce; SP5, stewed pork in water, salt, spices, soy sauce and sugar; SP6, stewed pork in water, salt, spices, soy sauce, sugar and cooking wine. LOP, Lipid oxidation products; AADP, Amino acid degradation products; MRP, Maillard reaction products; VFAB, Volatiles from aged brine; VFRM, Volatiles from raw meat; UO, Unknown origin. 103](#_Toc44494546)

[**Figure 4 - 3:** Score plots of PCA of the fresh and stewed pork. (a) PC1 plotted against PC2 and (b) PC1 against PC3. FP, fresh pork; SP1, stewed pork with water; SP2: stewed pork in water and salt; SP3: stewed pork in water, salt and spices; SP4: stewed pork in water, salt, spices and soy sauce; SP5: stewed pork in water, salt, spices, soy sauce and sugar; SP6: stewed pork in water, salt, spices, soy sauce, sugar and cooking wine. 108](#_Toc44494547)

[**Figure 4 - 4:** **(a)** Loading biplot of t1 and t2 of the model performed after PLS-DA of the volatile compounds in different pork samples. **(b)** Heat map of the correlations between volatile compounds and the pork samples. 111](#_Toc44494548)

[**Figure 4 - 5:** The odour sensory profiles of the fresh and stewed pork. 112](#_Toc44494549)

[**Figure 4 - 6: (a)** PLSR loading for the odour attributes and the odour-active compounds of the fresh and stewed pork. **(b)** Heat map illustrating the Pearson correlation between descriptor intensities and proportion. 116](#_Toc44494550)

[**Figure 5 - 1:** The processing flow chart of stewed pork. a-b: traditional stewing, c: high-temperature stewing. ……..127](#_Toc44494551)

[**Figure 5 - 2:** Response values of ten sensors **(a)** and PCA chart **(b)** of volatile flavour compounds in stewed pork with E-nose. The sensory of 10 chemical sensors are W1C (aromatic), W5S (broad-range), W3C (aromatic), W6S (hydrogen), W5C (arom-aliph), W1S (broad-methane), W1W (sulfur-organic), W2S (broad-alcohol), W2W (sulph-chlor) and W3S (methane-aliph). TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction. 136](#_Toc44494552)

[**Figure 5 - 3:** PCA score plot of E-tongue data for stewed pork with different processing methods. TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction. Taste sensor: AHS (to detect sour taste), SCS (to detect bitterness), PKS (to detect complex taste), ANS (to detect sweetness), NMS (to detect umami taste), CPS (to detect complex taste), CTS (to detect salty taste). 141](#_Toc44494553)

**List of Tables**

[**Table 2 - 1:** Ingredient composition of different marinades based on the product labels. 23](#_Toc44494568)

[**Table 2 - 2:** Definitions of odour attributes and reference standards. 25](#_Toc44494569)

[**Table 2 - 3:** Odour descriptions, odour thresholds and relative concentrations of volatile compounds in stewed pork by GC-MS/O. 28](#_Toc44494570)

[**Table 2 - 4:** OAVs of odour-active compounds in stewed pork. 33](#_Toc44494571)

[**Table 3 - 1:** Performance description and sensitivity of metal oxide sensors for PEN3 electronic nose………………..52](#_Toc44494572)

[**Table 3 - 2:** Concentration ratios and quantities of volatile composition of boiled pork from different breeds of pigs. 54](#_Toc44494573)

[**Table 3 - 3:** Identification and quantification of volatile compounds in boiled pork from different breeds of pigs by GC-MS/O (μg·kg-1). 57](#_Toc44494574)

[**Table 3 - 4:** Odour-active compounds (OAVs > 1) in boiled pork from different breeds of pigs. 61](#_Toc44494575)

[**Table 3 - 5:** Information of PCA, PLS-DA and one-way analysis of variance. 65](#_Toc44494576)

[**Table 4 - 1:** Information of the definitions and reference standards of odour attributes…………………………………83](#_Toc44494577)

[**Table 4 - 2:** Identification of volatile compounds of the fresh and stewed pork by GC-MS/O and GC×GC-TOFMS. 85](#_Toc44494578)

[**Table 4 - 3:** The comparison of kinds and content ratios of volatile components by GC-MS/O and GC × GC-TOFMS. 91](#_Toc44494579)

[**Table 4 - 4:** Concentrations and origin of volatile compounds of the fresh and stewed pork. 95](#_Toc44494580)

[**Table 4 - 5:** Odour-active compounds (OAVs > 1) in the fresh and stewed pork. 105](#_Toc44494581)

[**Table 5 - 1:** Performance description and sensitivity of metal oxide sensors for PEN3 electronic nose………………128](#_Toc44494582)

[**Table 5 - 2:** Program of gradient conditions of free amino acids. 129](#_Toc44494583)

[**Table 5 - 3:** The concentrations and types of volatile components in stewed pork with different processing methods. 131](#_Toc44494584)

[**Table 5 - 4:** Odour-active compounds (OAVs > 1) in different stewed pork. 133](#_Toc44494585)

[**Table 5 - 5:** Free amino acid contents (mg/100g) and taste threshold of stewed pork with different processing methods. 138](#_Toc44494586)

[**Table 5 - 6:** Nucleotide contents (mg/100g), taste threshold and EUC of stewed pork with different processing methods. 139](#_Toc44494587)

[**Table 5 - 7:** Concentrations (mg/kg) of fatty acid in stewed pork with different processing methods. 140](#_Toc44494588)

[**Table 6 - 1:** comparison among preparation techniques commonly used in food flavour analysis…………………….155](#_Toc44494610)

[**Table 6 - 2:** Characteristics of different analytical methods used in detection of flavour compounds. 157](#_Toc44494611)

**List of Abbreviations**

OAV, odour activity value

IMF, Intramuscular fat

SHS, Static headspace

DHS, Dynamic headspace

PT, Purge and trap

SPE, Solid phase extraction

SBSE, Stir bar sorptive extraction

SDE, Simultaneous distillation and extraction

E-nose, Electronic nose

E-tongue, Electronic tongue

DHM, Dahongmen,

DXC, Daoxiangcun

HHT, Henghuitong

TFH, Tianfuhao

EI, Electron ionization

NIST, National institute of standards and technology

IS, Internal standard

EDU, Enrichment and desorption unit

SD, Standard deviation

ANOVA, Analysis of variance

PCA, Principal component analysis

PLS-DA, Partial least squares-discriminant analysis

TB, Tibetan

SMX, Sanmenxia

DLY, Duroc × (Landrace × Yorkshire)

AHC, Agglomerative hierarchical clustering

LDA, Linear discriminate analysis

USDA, United States Department of Agriculture

VIP, Variable importance in the projection

RI, Retention index

PLSR, Partial least squares regression

FP, Fresh pork

SP1, Stewed pork with water

SP2, Stewed pork in water and salt

SP3, Stewed pork in water, salt and spices

SP4, Stewed pork in water, salt, spices and soy sauce

SP5, Stewed pork in water, salt, spices, soy sauce and sugar

SP6, Stewed pork in water, salt, spices, soy sauce, sugar and cooking wine

LOP, Lipid oxidation products

AADP, Amino acid degradation products

MRP, Maillard reaction products

VFAB, Volatiles from aged brine

VFRM, Volatiles from raw meat

UOAC, Unknown origin

PC1, First principal component

PC2, Second principal component

PC3, Third principal component

VID, Variable identification

TS, Traditional stewing

TSE, Traditional stewing with enzymatic degradation

TSE, Traditional stewing with enzymatic degradation and Maillard reaction

HS, High-temperature stewing

HSE, High-temperature stewing with enzymatic degradation

HSEM, High-temperature stewing with enzymatic degradation and Maillard reaction

UAAs, Umami amino acids, SAAs, Sweet amino acids

BAAs, Bitter amino acids

FAAs, Free amino acids

FAs, Fatty acids

HPLC, High performance liquid chromatograph

EUC, Equivalent umami concentration

MSG, Monosodium glutamate

RUC, Relative umami concentration

FAMEs, Fatty acid methyl esters

TAVs, Taste activity values

C16:0, Palmitic acid

C18:0, Stearic acid

C17:1, Ginkgolic acid

C18:1, Oleic acid

C18:2, Linoleic acid

C20:4, Arachidonic acid

SFAs, Saturated fatty acids

PUFAs, Polyunsaturated fatty acids

MUFAs, Monounsaturated fatty acids

SPME-GC-MS, Solid phase microextraction-gas chromatography-mass spectrometry

GC-MS/O, Gas chromatography-olfactomerty-mass spectrometry

GC × GC-TOFMS, Two-dimensional gas chromatography combined with time-of-flight mass spectrometry

1DGC-MS, One-dimensional gas chromatography mass spectrometry

GC × GC/HR-TOFMS, Two-dimensional gas chromatography with high-resolution time-of flight mass spectrometry

GC-O, Gas chromatography-olfactometry

DVB/CAR/PDMS, Divinylbenzene/carboxen/polydimethylsiloxane

**1**

# Chapter Ⅰ. General Introduction

# Context

The traditional stewed pork is processed by stewing the hind leg meat in aged brine (water, salt, sugar, spices, soy sauce, cooking wine and other condiments) for a long time. With the simple processing techniques, sauce pickled meats are very common in China. The stewed pork products are appreciated by consumers owing to the unique aroma and taste profile. Flavour is the most important factor to determine the meat character and purchasing decision of the consumers (Reicks et al., 2011). The flavour refers to the smell of fresh meat, and the aroma and taste of meat products after heating. It is caused by complex biochemical changes in the inherent components of meat, resulting in various organic compounds. The flavour compounds in meat products consist of volatile compounds and non-volatile taste compounds (Dashdorj, Amna, & Hwang, 2015; Kosowska, A. Majcher, & Fortuna, 2017). The volatile compounds are mainly odorant compounds, which are mainly felt by human olfactory cells and transmitted to the brain via nerves to produce aromatic sensations. The non-volatile compounds are taste compounds, mainly relying on the taste sensation of human tongue bud, through nerve conduction to the brain to reflect the taste. More than 1000 volatile compounds have been identified in the meat products, including aldehydes, alcohols, aliphatic hydrocarbons, aromatic hydrocarbons, ketones, carboxylic acids, esters, lactones, sulphur compounds, nitrogen compounds, and other heterocyclic compounds (Jayasena, Ahn, Nam, & Jo, 2013; Kosowska et al., 2017; Shahidi, 1998). Taste compounds are mainly free amino acids, nucleotides and small peptides in meat products (Xu, You, Song, Gong, & Pan, 2018). Odour compounds and taste compounds affect the final flavour formation of meat products.

The research on the flavour of meat products began in the 1950s. It is believed that there are two main types of meat flavour precursors: water-soluble compounds (such as amino acids, peptides, carbohydrates, nucleotides, thiamine, etc.) and fat-soluble compounds. At that time, it was mainly to identify the non-volatile water-soluble precursors of meat flavour, and the analysis of meat aroma was still at the level of sensory analysis. In the 1960s and 1970s, people began to pay attention to volatile flavour substances in meat. It was found that under the use of barbecue, cooking and other processing methods, a series of chemical reactions will occur in non-volatile compounds in lean meat and fat tissue. These reactions produce many volatile compounds. In 1978, the first international conference on flavour research was held at the University of East Anglia in the United Kingdom. Later, the gas chromatography mass spectrometry (GC-MS) were applied for the detection of volatile flavour substances in cooked meat products.

The threshold of volatile aroma compounds is the lowest concentration of the olfactory sensor when it was sensed odour, and it is an important indicator to measure the activity of aroma compounds. Some high levels of volatile flavour compounds may contribute little to flavour (high threshold), and some low levels of volatile compounds may contribute significantly (low threshold). For example, the odour threshold of lipid compounds is higher than the sulphur- and nitrogen-containing heterocyclic compounds. In 1963, Rothe and Thomas calculated the ratio of the concentration of flavour compounds to the odour threshold, and expressed as "aroma value". This method was the first attempt to evaluate the contribution of single compound to the overall flavour of food. Since then, some similar methods have been found: the odour activity value (OAV) was used to evaluate the effect of volatile aroma compounds. When the OAV of volatile substances is greater than or equal to 1, it may have a direct effect on the aroma. When the OAV is less than 1, volatile substances may have no actual effect on the total flavour or have an indirect effect (synergy, antagonism) (Liu, He, & Song, 2018). These compounds that contributed to the flavour of food are called odour-active compounds (Carrapiso, Ventanas, & García, 2002). Some compounds that had the most contribution to flavour were considered the key odour-active compounds (Guth, 1997).

The stewed meat products are typical representatives of traditional Chinese meat products with a history of more than 3,000 years, which is the largest meat product in China. However, compared with Western-style meat products, we find that the development of traditional stewed meat products still faces many difficulties and bottlenecks. The traditional stewing method is not suitable for industrialization and standardized production due to large cooking loss, serious nutrition loss and unstable flavour quality. Among them, the unstable flavour quality was one of the main problems. Therefore, it is necessary for stability and improvements of traditional stewed pork flavour.

# Objective

The first aim of the thesis was mainly to characterize volatile compounds in stewed pork and differentiate stewed pork from four local brands. The second aim was to assess the influence of different breeds of pigs on the flavour compounds in boiled pork. The third aim was to investigate the effect of different seasoning recipes on volatile profiles and sensory evaluation of stewed pork. The fourth aim was to analyse the volatiles and non-volatiles profile of stewed pork with different processing methods to improve the flavour of stewed pork. Therefore, the literature review and four main experiments were designed, validated and completed in the follow-up work.

# Research strategy

The literature review introduces the work undertaken in this thesis by understanding the volatile profile of pork to improve the flavour of traditional stewed pork. This review reported on the formation mechanism of stewed meat products and the factors that affected the flavour of meat products, discussed the extraction, separation and identification technology of volatile flavour components of the meat products.

It was analysed volatile compounds profile of Chinese stewed pork from four local brands and confirmed the potential flavour marks (Chapter 2) and evaluated the effects of different breeds of pigs on the flavour compounds in boiled pork (Chapter 3). The results of the experiments were reported in Chapter 2 and Chapter 3 and were published in Molecules and Food Research International. It also investigated the effluence of different seasoning recipes on volatile profiles and sensory evaluation of stewed pork (Chapter 4), the volatile compounds and taste compounds profile of stewed pork with different processing methods (Chapter 5). Chapter 4 and 5 has been prepared to submit in Food Chemistry and Journal of the Science of Food and Agriculture. Then the thesis was finalized by a general discussion leading to discussion, conclusions and perspective (Chapter 6).

# Literature review

## *4.1. Overview of stewed meat products*

The traditional stewed meat products were produced by boiled, cooked and soaked steps (Zeng et al., 2016). Firstly, the fresh or frozen meat were boiled in water for 5 min to remove the blood, and then stewed in aged brine that has been prepared with salt, soy sauce (or not added) and spices for 45 min, finally soaked for 60 min. According to different processing techniques, the stewed meat products are divided into soy sauce-stewed meat, boiled meat and vinasse-stewed meat, etc. For different flavour characteristics, they are also divided into braised meat, spicy meat, soy sauce-stewed meat, sweet and sour-stewed meat, etc. The flavour compounds in stewed meat products can generally be divided into two categories, namely odour compounds and taste compounds. During the heating of meat, the precursor substances undergoes a series of changes, such as the degradation of sugars, peptide amino acids, and vitamins, the oxidative dehydration and decarboxylation of lipids and fatty acids, and the Maillard reaction of reducing sugars and amino acids (Shahidi, F., 1994; Mottram, D. S., 1998; Aaslyng & Meinert, 2017). A series of complex and volatile flavour substances were formed the flavour of stewed meat products.

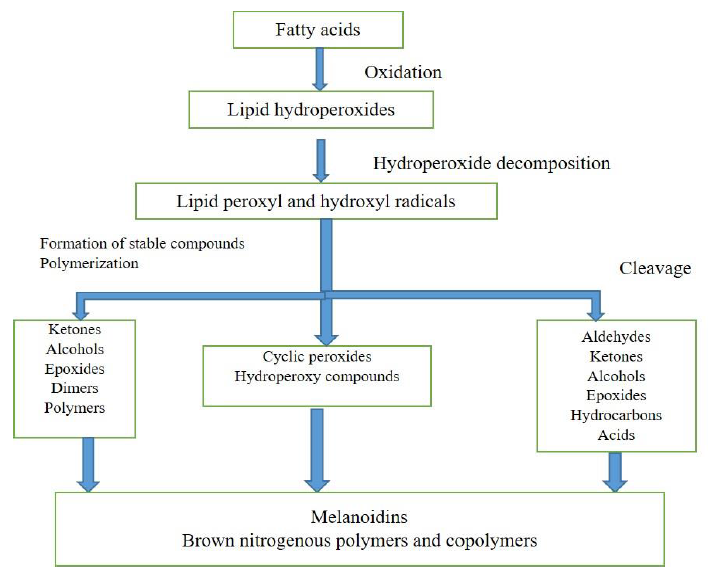
## *4.2. Formation paths of flavour compounds*

### 4.2.1. Thermal degradation reaction

The thermal reaction process has a certain role in promoting the formation of meat flavour. When the temperature is at a high state, many flavour precursors of meat would undergo degradation reaction, producing a large number of primary degradation products in the form of reaction intermediates. These products will continue to react to produce characteristic aroma compounds. Cerny, (2007) analysed the aroma substances generated by the Maillard reaction system of xylose, cysteine and thiamine, and found that the cysteine would undergo Strecker degradation reaction during heating, resulting in the intermediate of ammonia, H2S, etc. These intermediate have high reactivity and can provide a rich source for sulphur-containing compounds. It has also been found that if the reaction system lacked cysteine, the sulphur-containing compounds were usually derived from thiamine, xylose had little effect on the formation of various volatile compounds in the reaction system. Thus, it was confirmed that there is the important role of degradation products of cysteine for the entire reaction mode. In addition, under higher temperature conditions, the sugar would undergo caramelization and degradation reactions, the former of which leads to the formation of furfural form pentose and hydroxymethylfurfural from hexose. The furan derivatives, carbonyl compounds and alcohols would be produced when the heating continued, the latter would degrade sugar to form dicarbonyl and tricarbonyl compounds. The intermediated for the reaction could undergo Strecker degradation reaction with amino acids to generate to a large amount of flavour compounds (Van den Ouweland & Peer, 1975).

### 4.2.2. Lipid oxidation

Lipid is a group of important chemicals in the food and food products. It plays an important role in the price of products, texture, flavour, and the accessibility of food products by consumers (Diez-Simon, Mumm, & Hall, 2019). However, lipids can provide the foods positive or negative qualities depending on the type of food, in which it is responsible about rancid flavour in the milk (negative effect), and in cheese, they are considered as the major flavour component (positive effect) (Diez-Simon et al., 2019). During food processing such as heating, cooking, or frying, fatty acids are subject to autoxidation to form hydroperoxides, which further undergo decomposition and produce peroxyl and hydroxyl radicals (generally oxidation of fatty acid occurred by three consecutive stages including, initiation, propagation, and termination). Peroxyl and hydroxyl radicals will rapidly undergo the cleavage in many routes to produce many volatile and non-volatile compounds such as alcohols, ketones, aldehydes, acids, dimers, polymers, and epoxides (Diez-Simon et al., 2019; Frankel, 2014) (see Figure 1 - 1).



**Figure 1 - 1:** The mechanism of flavour formation through the lipid oxidation.

Adapted from (Diez-Simon et al., 2019).

### 4.2.3. Maillard reaction

The Maillard reaction is a non-enzymatic browning reaction, which is essentially a series of complex chemical reactions that occur between carbonyl compounds and amino compounds under the effects of dehydration, cracking, condensation, and polymerization (Mottram, D. S. 1994). The classic Maillard reaction has three reaction stages: initial stage, middle stage and Strecker degradation reaction stage (Mottram, D. S. 1998). Strecker degradation is that 3-Deoxyglucosone reacts with amino acids to form aldehydes and aldehydes and enols, which are then dehydrated and cyclized to form pyrazine derivatives. The aroma formed during the processing of roasted meat is mostly produced by pyrazines (Chen, Song, & Ma, 2009). The primary stage of the Maillard reaction is mainly via the nucleophilic addition reaction between the carbonyl carbon of the reducing sugar and the free amino nitrogen, loss of water to form a glucosamine, and then by Amadori or Henys rearrangement to form various flavour precursors (such as aminodeoxyketone, Amadori rearrangement compounds and Henys rearrangement compounds, etc.); the middle stage is to further degrade various precursor substances formed in the primary stage to produce substances such as furfural, furanone, etc. These substances would react with ammonia and amino acids. Many important flavour substances are formed by this stage reaction, such as thiophene, thiazole, pyrazine and other heterocyclic compounds; Strecker degradation reaction stage is mainly the process of deamination and decarboxylation of amino acids in the presence of diketone compounds. The degradation products (such as aminoketone) formed at this stage are important intermediate reaction substances to form pyrazine, thiazole and other heterocyclic compounds (Calkins & Hodgen, 2007; Namiki & Hayashi, 1983; Van Boekel, 2006).

The results of the pervious reports have fully showed that although many meat flavour compounds can be produced by the thermal degradation reaction of thiamine or a mixture of reducing sugars and ammonia, and the Maillard reaction was still recognized as an important reaction for the formation of meat flavour. The flavour compounds produced by the Maillard reaction are affected by multiple factors. These factors can be divided into two categories, namely, the characteristics of the reaction base and the reaction conditions. Changes of the reaction base and conditions would cause the formation of flavour compounds to migrate, which obviously effected flavour quality of the final product. Therefore, it is very important for the formation of flavour of the final product by selecting the appropriate reaction precursor and content (Amrani-Hemaimi, Cerny, & Fay, 1995; Madruga & Mottram, 1995; Shibamoto & Russell, 1976). Baek, Kim, Ahn, Nam, & Cadwallader, (2001) has added appropriate amounts of sulphur-containing amino acids and thiamine under the conditions of enzymatic hydrolysis of plant protein, and successfully simulated the flavour of beef through the model Maillard reaction system.

### 4.2.4. Lipid-Maillard interactions

The process of lipid thermal reaction could promote the flavour formation of food. The study has showed that meat flavour was related to water-soluble flavour precursors, while the characteristic flavour of different meats was related to lipids (Mottram, D. S. 1998; Umano & Shibamoto, 1987; Zamora & Hidalgo, 2005). Pearson et al., (1973) found that the flavour of extracted liquid of free-fat beef and lamb was similar and difficult to distinguish by sensory analysis, however if the butter and goat oil were added, which showed their own distinctive flavour. The various flavour compounds produced by lipids during heating, some of which are derived from oxidative degradation of lipids, such as fatty aldehydes, fatty alcohols and fatty ketones. These products of oxidative degradation were formed the characteristic flavour of meat. Another part of compounds is the fat oxidation products that could be used as reaction precursors. Which has the competition mechanism with the amino acid in Maillard reaction. The pathway of original reaction products was changed to promote the generation of new aroma compounds, it helps to balance various aroma compounds and plays the modification role in product flavour (Elmore, Mottram, Enser, & Wood, 1997; Mottram & Edwards, 1983; Song et al., 2012).

Xu et al., (2011) analysed the volatile compounds formed by the Maillard reaction system of cysteine-xylose with no added oil, unoxidized and oxidized lard. It is found that the addition of lard has a greater effect on most sulphur-containing compounds. Maarse, & van der Heij, (1994) found that the presence of lipids could cause changes in the number and type of Maillard reaction products, and at the same time, the synergistic effect of at oxidation products and Maillard reaction produced some new products. Whitfield, & Mottram, (1992) also found that there was a large number of long-chain alkyl thiophene, formylthiophene, pyridine, nitrogen-, oxygen- or sulphur-containing heterocyclic compounds in the presence of lipids or fatty acids, where the alkyl group was usually derived from aliphatic aldehydes (mainly obtained by lipid oxidation), and amino acids provided nitrogen and sulphur sources. These studies have confirmed the above points, the alkylthiazoles with C4-C8 in the 2-position have been identified in roast beef (Hartman et al., 1983) and fried chicken (Tang, Jin, Shen, Ho, & Chang, 1983). Among the volatile components of cooked beef, the alkylthiazoles with longer 2-alkyl substituents (C13-C15) and alkyl-3-thiazolines have also been found (Elmore & Mottram, 1997).

## *4.3. Factors influencing formation of flavour compounds*

### 4.3.1. Breeds of meat

The composition of pork is very complex, mainly including water, protein, amino acids, sugars and lipids, etc. These ingredients have an important role in the flavour of pork and could be used as flavouring substance, flavour precursor and flavour enhancer (Khan, Jo, & Tariq, 2015). Taste mainly comes from flavouring substances in meat, such as inorganic salts, amino acids, peptides, acids and sugars. After being sensed by the taste buds on the tongue, these substances are transmitted to the brain through the nerves, thereby showing sweet, salty, sour, bitter, fresh and other tastes (Dashdorj et al., 2015). Meat flavour precursors are mainly divided into water-soluble compounds and fat-soluble compounds. Water-soluble compounds include sugars, free amino acids, peptides, nucleotides and thiamine (Khan et al., 2015). Glucose, glucose-6-phosphate, ribose, ribose phosphate, and fructose could increase the flavour of roasted caramel. The addition of ribose and glucose could increase the level of volatile compounds in pork, of which alkyl pyrazine is the most abundant (Neethling, Hoffman, & Muller, 2016). Degradation products such as ammonia, hydrogen sulfide, methyl mercaptan, and methyl thioaldehyde produced by the thermal decomposition of sulfur-containing amino acids have the low odour threshold, and their low amount could increase the aroma of cooked meat (Shahidi, Rubin, & D'Souza, 1986). Fat-soluble compounds are represented by fatty acids. The composition of fatty acid has a great influence on flavour components (Dashmaa et al., 2011; Song et al., 2017). Due to the different fatty acid composition of pork from different varieties and different degrees of oxidation, the flavour compounds produced are also different. The muscle fatty acid content of various breeds of pigs had a similar trend that were the higher content of oleic acid and palmitic acid, followed by linoleic acid and stearic acid, and lower content of myristic acid and linolenic acid.

As reported, there were 118 Chinese native pigs according to the Domestic Animal Diversity in the word index (Scherf, 2000). For example, Tibetan pig is known as a unique pig breed in the Qinghai-Tibet Plateau region of China that are characterized by stronger disease resistance and higher meat quality than other native pigs (Mi et al., 2019). Sanmenxia pork, as a local specialty food, has become increasingly appreciated by consumer due to its favourable organoleptic properties, high protein and amino acid contents (Shen et al., 2014). As a typical hybrid pig, Duroc × (Landrace × Yorkshire) pig is now widely used for commercial production and the texture and flavour of this pork is notably different from that of local pork (Ji et al., 2018).The research had shown that the breed of pork is greatly impacted on intramuscular fat (IMF), tenderness, juiciness, especially flavour (Lu, Li, Yin, Zhang, & Wang, 2008; Meinert, Christiansen, Kristensen, Bjergegaard, & Aaslyng, 2008). Compared with the hybrid pigs, the cooked pork from Chinese indigenous breed pigs had higher flavour intensity (Lu et al., 2008). In summary, these analysis showed that different types of pork have different flavours due to the differences of flavour precursors in the meat. Even for the same kind of pork, the difference of the flavour compounds in different breeds may be significant.

### 4.3.2. Spices

Spices are natural botanical seasonings that have both fragrant and spicy flavour, as well as hemp, spicy, bitter, sweet and other flavour (Raghavan, 2007). In addition to colouring and flavour enhancing, the spices also have antibacterial, antiseptic, and strong antioxidant effects. During the processing of stewed meat products, the spices could impart the unique flavour, enhance and improve the flavour of meat products. It could also suppress and correct the bad smell in stewed meat, highlight the flavour characteristics. The main functions in the processing of stewed meat are as follows: (1) It has the functions of processing raw materials, masking odours and generating special flavours (Sun, Chen, Li, Liu, & Zhao, 2014). Zanthoxylum, cinnamon, pepper and cloves could enhance aroma and suppress off-odour in stewed meat products. Licorice and grass fruit could give the meat the rich and spicy aroma, and make people have aftertaste effect. (2) It has anti-oxidation function (Mancini, Paci, Dal Bosco, Mattioli, & Preziuso, 2019). Many spices can be used as raw materials for extracting natural antioxidants. (3) It has antibacterial and antiseptic functions (Cao et al., 2013). In order to protect themselves from bacteria, fungi and insects, the spice plant has formed unique smell and taste. The simultaneous use of several spices can significantly extend the shelf life of stewed meat. (4) It has physiological and pharmacological functions (Ghasemzadeh, Jaafar, & Rahmat, 2015). Most spices are components of traditional Chinese medicine prescriptions and play an important role in the formulation of traditional Chinese medicine.

### 4.3.3. Cooking method

Cooking method plays a vital role in the formation of meat flavour, which affects the acceptability and volatile flavour components of meat (Sañudo et al., 2000). Christensen et al., (2012) stored the heat-treated chicken at low temperature for long-term storage and evaluated its sensory characteristics. It was found that long-term treatment at low temperature had little effect on the flavour of chicken meat, and time had a greater effect on the flavour intensity of cooked meat. This showed that the intensity of flavour compounds was lower at low temperatures, because they are mainly produced at higher temperatures. The cooking methods such as baking, grilling and frying, high-pressure cooking and boiling, as well as include heat treatment at temperatures above 100 °C. Which were conducive to the formation of a large number of heterocyclic compounds in cooked meat (MacLeod, 1986). High temperature and low humidity conditions are required for the formation of pyrazine compounds. Therefore, many pyrazine, pyridine, pyrrole and thiazole compounds were only found in roasted chicken and fried chicken, but not in chicken broth (Aliani & Farmer, 2005). The different pre-treatments of cooking also effect the meat flavour. The addition of glucose of broiled pork neck with edible acetic acid produces more volatile aldehydes, carboxylic acids, esters, furans, pyrans, pyrazines, and pyrrolopyridine derivatives than no added glucose (Biller, Boselli, Obiedziński, Karpiński, & Waszkiewicz-Robak, 2016).

## *4.4. Extraction methods of flavour compounds*

### 4.4.1. Headspace extraction

The headspace analysis technology consists of many solvent-free extraction techniques, which separated volatile components from the food matrix. This technology is to place the solid (liquid) sample in a container with the certain head space, and collects upper air in equilibrium. The components of headspace gas in food were olfactory compounds produced by stimulating human olfactory cells, which represent the true flavour of food. Therefore, the headspace analysis is one of the most widely used methods for food flavour analysis. It generally consists of static and dynamic headspace methods.

The static headspace (SHS) is to put the sample in a closed container, and then directly get headspace gas for sampling, after the thermodynamic dynamic balance is reached between the sample and the surrounding headspace. This technology has developed into the mature technology in the 1960s and has been widely used (Robbins, Wang, & Stuart, 1993). Robbins et al., (1993) has identify 67 volatile compounds in bitter beans using SHS-SPME-GC-MS technology, and found that ultrasound, water bath temperature, extraction time and other factors have affected the extraction efficiency. The advantage of the SHS method is that the sample preparation is simple, no solvent is used, and there is no problem of cross-contamination. The use of automatic sampling technology can provide high repeatability, operability and sensitivity. However, this method is only suitable for the detection of highly volatile and high content of volatile components.

Dynamic headspace (DHS), also known as purge and trap (PT), was the second breakthrough in the development of headspace technology and appeared in the 1970s (Liebman, & Levy, 1983). This method uses an inert gas stream (generally high-purity nitrogen) to pass through the sample to take out the volatile compounds, and then uses a trap tube equipped with an adsorbent to adsorb and extract the volatile flavour substances in the gas stream. DHS has the characteristics of low sampling volume, high enrichment efficiency, small matrix influence and easy online monitoring. It is widely used in the extraction of volatile flavour substances of various food. Resconi et al., (2010) studied the effect of different feeding conditions on the flavour of lamb using and dynamic headspace-solid phase extraction (DHS-SPE) technology based on the features of high extraction efficiency and sensitivity of DHS technology. The extraction conditions of DHS are relatively mild, and some flavour components with relatively low thermal sensitivity can be obtained, and the purged inert gas would not pollute the extracted flavour substances. However, this method had cross-contamination during solvent desorption, the dynamic headspace technology is often used in conjunction with adsorption extraction to extract flavour components. Pontes, Pereira, & Câmara, (2012) analysed the volatile components of five bananas by dynamic headspace-solid phase microextraction-gas chromatography-mass spectrometry (DHS-SPME-GC-MS), and found that this method could solve the cross-contamination problem well.

### 4.4.2. Adsorption extraction

Adsorption refers to the adsorption of gas or solute on the solid or liquid surface, that is, the concentration of solute components at the interface of the mobile phase and the stationary phase, which is divided into physical adsorption and chemical adsorption. Combined with the simplification, miniaturization, easy operation, low sample volume, and the green analysis principle of reducing or not using organic solvents, this kind of separation and extraction technology is relatively gentle. It has developed rapidly in recent years, especially for trace analysis, these extraction techniques based on adsorption-desorption are suitable for the extraction of volatile and semi-volatile compounds from almost all food sample matrices (Tobiszewski, Mechlińska, Zygmunt, & Namieśnik, 2009). In addition, the extraction technologies generally have high-throughput characteristics, which are convenient for connecting high-selectivity and high-sensitivity detection systems (Olariu, Vione, Grinberg, & Arsene, 2010; Raynie, 2006). Among them, solid-phase microextraction and stir bar adsorption extraction are typical applications of adsorption extraction technology for volatile flavour components in food.

Solid phase micro-extraction (SPME) is a fast, solvent-free pre-treatment technology of test samples. This technique applies 0.6-0.9 mg adsorbent on the optical fibres to achieve the extraction of volatile components of the sample matrix, provide energy to complete desorption and injection through the chromatographic inlet (Bicchi, Cordero, Iori, Rubiolo, & Sandra, 2000; Bicchi, Cordero, Liberto, Rubiolo, & Sgorbini, 2004). The SPME extraction process includes two steps: separating the target compound from the sample matrix; and desorbing the target compound to the analytical instrument (Risticevic, Niri, Vuckovic, & Pawliszyn, 2009). The SPME is still considered to be the better preparation technology in the past 20 years (Arthur & Pawliszyn, 1990), and it has been rapidly applied and promoted in analytical chemistry, environment, food flavour, and clinical (Ho, Canestraro, & Anderson, 2011).

The rapid development of SPME is mainly based on the following points: First, the operation is fast, simple and solvent-free. Second, the selectivity of the fibre coating allows SPME to be applied to a wider range of analysis fields. Third, it is easy to realize automated sampling. SPME and three-dimensional auto-sampler can be used on almost all types of gas chromatographs. Thermo Fisher SPME auto-sampler could be connected to two independent GC systems to achieve heating, extraction, desorption and cleaning automation, which greatly improved the analytical capabilities of SPME, and has higher reproducibility and analysis efficiency than manual sampling (Ho et al., 2011). In addition, the protective coating makes the adsorbent less contaminated, so SPME can not only extract the compounds from headspace gas, but also directly extract volatile and semi-volatile components in liquid samples (Zhang, Poerschmann, & Pawliszyn, 1996). The stirring state and temperature of samples, and the state of the fibre coating (pollution and humidity) had a significant impact on the results, due to the sensitivity of SPME.

The highly volatile compounds were not extracted by SPME. Horák et al., (2009) analysed free fatty acids in beef using the methods of SPE, SPME and stir bar sorptive extraction (SBSE), and found that the fatty acids (C4:0-C12:0) were extracted and highly volatile or unstable compounds (C1:0-C4:0) were not suitable for extraction. Because non-volatile components such as sugars, fats, proteins, and pigments usually contained in the food matrix would contaminate the adsorbent, the SPME method was usually used in food analysis. Xiao et al., (2014) investigated the changes of volatile components before and after roasting almonds by HS-SPME-GC/MS. A total of 58 volatile components were identified. It was found that linear aldehydes and alcohols are the most important volatile compounds in raw almonds and roasted almonds.

### 4.4.3. Simultaneous distillation and extraction (SDE)

Simultaneous distillation and extraction (SDE) are an effective method for extracting, separating and enriching volatile and semi-volatile flavour components in food. This method combines steam distillation and solvent extraction, so that the water vapour containing the sample components and the extraction solvent vapour are fully mixed in the device. After condensation, the two phases are fully contacted to achieve the phase transfer of the components, to achieve efficiency extraction, reduce experiment steps and shorten analysis time. Over the past 20 years, SDE has been widely used as the pre-treatment method for gas chromatography, gas chromatography-mass spectrometry, liquid chromatography and other analytical experiments. The SDE method, using methylene chloride as a solvent, is widely used for the extraction of volatile flavour compounds from fish (Morita, Kubota, & Aishima, 2003). For example, Selli, & Cayhan, (2009) has used methylene chloride as a solvent, extracted and identified 46 flavour compounds from gilthead sea bream by SDE combined with GC-MS.

The SDE method is widely used in the extraction of flavour substances of food because of its simple operation, good repeatability and high recovery rate. However, due to the high temperature operation, the extraction of low vapour pressure components is difficult, and it is easy to destroy some heat sensitive compounds, or volatile components not contained in the sample itself could produce during the extraction process. For example, Riu-Aumatell et al., (2011) found that the SDE method could identify more macromolecular terpenes, acids and esters than headspace solid phase micro-extraction (HS-SPME). This was because the flavour components had changed during the distillation process. In addition, when the solvent was removed after completing SDE, the solvent would take away some flavour components, which affects the accuracy of the analysis results. It can be seen that the extraction efficiency of SDE is mainly affected by the solvent, salt, distillation time, oxygen and distillation temperature.

## *4.5. Analysis of flavour compounds*

### 4.5.1. Gas chromatography-olfactometry-mass spectrometry (GC-MS/O)

GC-MS had been widely applied to food aroma analysis as a prevalent analytical technique, due to its ability to separate and identify volatile compounds (Chen, Song, Bi, Meng, & Wu, 2018; Feng et al., 2011; Lorenzo & Fonseca, 2014). Li et al., (2016) applied the GC-MS method for the comparison of volatiles in Chinese marinated drumsticks produced from different marinating methods, and a total of 44 and 60 volatile flavour compounds were identified, respectively. Sandra, Nives, Katarina, Eddy, & Helga, (2018) found 149 volatile compounds of dry-cured hams from different processing methods by GC-MS. Lou, Zhang, Sun, Wang, Pan, & Cao, (2018) showed that two kinds of vinasse-curing methods influenced the characteristic volatiles of products and their generation during processing. But it is difficult to distinguish the contribution of each single volatile to integral food aroma profile. So, the OAV is introduced. Which can be calculated based on dividing the concentration of a compound by its mean recognition threshold in matrix (Gu, Wang, Tao, & Wu, 2013; Zhou, Chong, Ding, Gu, & Liu, 2016). Moreover, one of the weakness of GC-MS method is that it could not associate the real sensory experience. Therefore, it is pointed out to link the sensory experience and chemical compounds by GC-O.

With strength in linking compound analysis with sensory evaluation, GC-O has been employed to investigate the aroma profiles of the samples. 36 aroma compounds were detected in five varieties of mandarins using GC-O (Xiao et al., 2017). The relationship was established between odour-active compounds identified by GC-O and flavour attributes form the sensory evaluation in cooked cured pork ham (Iu et al., 2016). GC-O-MS consists of two powerful working units, GC-O and GC-MS, which integrate the characteristics of these two devices into an integrated instrument (Song, & Liu, 2018). GC-MS and GC-O were simultaneously employed to obtain reliable data of the odorant composition. The combination of olfactory detector and gas chromatography-mass spectrometry is particularly effective for identifying or extracting aroma active compounds from various volatile components. The application of GC-O-MS has been extensively reported in various food products, such as chocolate (Liu et al., 2015), fish (Wu, Tao, & Gu, 2014) and tea (Luo, Chen, Gao, Liu, & Wu, 2017). Nowadays, the GC-O-MS method for the identification and quantification of the individual volatile compounds in the stewed pork have not been reported.

### 4.5.2. Two-dimensional gas chromatographic combined with time-of-fight mass spectrometry (GC × GC-TOFMS)

GC × GC combines two chromatographic columns with different separation mechanisms and independent from each other through a modulator to form a two-dimensional gas chromatography system. The modulator plays the role of collection, focusing and retransmission. Each peak separated by the first chromatographic column must enter the modulator and be pulsed to the second chromatographic column for the second separation. This is called full two-dimensional.

The traditional one-dimensional gas chromatography mass spectrometry (1DGC-MS) is a commonly used technique for the study of volatile components of food. However, due to the limited resolution of 1DGC, co-elution occurs when analysing a large amount of volatile substances in complex samples, which has an impact on qualitative and quantitative analysis. Combining a variety of pre-treatment methods, it could effectively separate complex samples before entering gas chromatography, but this also brings the problem that the process is complicated and difficult to quantify. GC × GC is a multi-dimensional chromatographic separation technology, which has high resolution, high sensitivity and large peak capacity. On the other hand, combined with high-sensitivity time-of-flight mass spectrometry (TOFMS), it is a powerful tool for the separation and identification of volatile components of complex samples (Tranchida, Franchina, Dugo, & Mondello, 2016), and has been widely used in analysis of the complex samples (Prebihalo et al., 2018), such as petroleum (Frysinger & Gaines, 2001), environmental samples (Focant, Sjödin, & Patterson Jr, 2004), and plant essential oils (Marriott, Shellie, Fergeus, Ong, & Morrison, 2000). In recent years, the reports had also showed that GC × GC was applied for flavour compounds analysis in meat products. For example, the comprehensive two-dimensional gas chromatography with high-resolution time-of flight mass spectrometry (GC × GC/HR-TOFMS) and GC-MS were used to identify the volatiles from dry-cured hams. It was shown that a total 165 volatile compounds were found by GC × GC/HR-TOFMS while only 50 compounds were found by GC-MS (Wang et al., 2018). Some trace sulphur and nitrogen-containing compounds that might play important roles in the flavour of braised chicken were only detected by the GC × GC/HR-TOFMS (Duan et al., 2015).

### 4.5.3. Electronic nose (E-nose) and electronic tongue (E-tongue)

Buck and DarVnieks et al. developed "electronic nose" using dour modulation conductance and modulation contact potential in 1965. An international academic conference, with E-nose as the main content, held by the North Atlantic Treaty Organization and defined the concept of E-nose in 1989: the E-nose is a device composed of multiple gas sensors with overlapping properties and appropriate pattern classification methods that had the ability to identify single and complex gas. Subsequently, the first E-nose academic conference was held in 1994 (Gardner, & Bartlett, 1994). Since then, E-nose technology has been widely studied by people. In order to promote the development of E-nose technology, the chemical sensor meeting is held annually in the world.

The E-nose is mainly composed of three parts (1) the headspace sampler: the gas above the sealed bottle with the sample is sucked into the sensor through the headspace; (2) the sensor: the odour acts on the sensor array to generate an instant response, the response is from strong to weak, and finally reach a stable state; (3) the signal processing system: the odour information obtained by the gas sensor array, that is, the pattern recognition system, which could pre-process and perform feature extraction, and then use the software to perform the system analysis. Thus, which could complete the detection and analysis of complex odours (Wilson, 2012). The E-nose is a sensor technology that simulated human olfactory sensation (Röck, Barsan, & Weimar, 2008). The sensor array is used to obtain the response signal of the analyst. The parameter model technology was used to process the response model into coordinates to form the fingerprint map, so as to obtain the difference between different odours, which could avoid the defect of physiological smell and ensure the repeatability and stability of the same sample. The overall information of the volatile components in the sample could be determined qualitatively and quantitatively by statistical methods of chemometrics (Benedetti, Buratti, Spinardi, Mannino, & Mignani, 2008; Gómez, Wang, Hu, & Pereira, 2006). At present, the E-nose has been widely used in the processing of meat products, such as discrimination of freshness of meat (Hong, Wang, & Hai, 2012), determination of adulteration of meat (Nurjuliana, Man, Hashim, & Mohamed, 2011), microbial monitoring in chilled meat (Wang, Wang, Liu, & Liu, 2012), and the effect of pig feeding methods on Iberian hams (Santos et al., 2004).

E-tongue is a liquid analysis instrument based on biological taste patterns, chemical sensors and pattern recognition. The sensor array in the electronic tongue system is equivalent to the tongue in the biological system. The function of each independent sensor is similar to the taste bud on the tongue surface, which could feel different chemicals. The overall taste characteristics were identified through data processing and mode of the computer. Many reports showed that the overall quality analysis and flavour identification of electronic tongues were applied to foods such as fruit juice, wine, braised meat and coffee (Dong, Zhao, Hu, Dong, & Tan, 2017; Haddi et al., 2014; Liu et al., 2017; Yu, Zhao, Li, Tian, & Ma, 2015), which is enough to reflect the application value of electronic tongue technology.

### 4.5.4. Chemometrics

Chemometrics is an interdisciplinary discipline that emerged in the 1970s and 1980s. It is based on the methods and principles of mathematics, statistics, chemistry and computer science, designing optimized experiments, mining and processing, resolving and parsing information of experimental measurement data to obtain useful information hidden in complex analysis systems (Kumar, Bansal, Sarma, & Rawal, 2014). The principal component analysis (PCA) (Yang et al., 2019), partial least squares-discriminant analysis (PLS–DA) (Pavlidis, Mallouchos, Ercolini, Panagou, & Nychas, 2019), agglomerative hierarchical clustering (AHC) (Lee, Kwak, Joo, Kang, & Lee, 2018) and partial least squares regression (PLSR) (Zhang et al., 2018) have been confirmed to be the most frequently used chemometric methods. Based on the concept of dimensionality reduction, PCA could transform multiple indicators into several comprehensive ones, thus simplifying the analysis process (Gutierrez-Osuna, 2002; Hidalgo, Pozzi, Furlong, Marchevsky, & Pellerano, 2018; Toraman et al., 2018). In PCA, the score chart showed the similarities and differences of various samples according to the style and content of volatile compounds (Petričević, Radovčić, Lukić, Listeš, & Medić, 2018). PLS–DA is a stable discriminant statistical method, especially suitable for cases with large numbers of explanatory variables, multicollinearity, small numbers of sample observations and large interference noise (Kumar & Sharma, 2018; Sharma & Kumar, 2018). The VIP of PLS–DA could quantify the contribution of each variable to the classification. The larger the VIP, the more significant the difference of the variable among boiled pork from different breeds (Han, Zhang, Fauconnier, & Mi, 2019). AHC is an exploratory technique used to group samples based on the types of similarity measures used. Samples with similar spectral signatures were most likely to form a cluster (Kumar & Sharma, 2018). Partial least squares regression (PLSR) may be the best way to clarify the correlations between multiple variables by reducing the dimensions of the original data set without losing any information (Lin & Zhang, 2016). It could also be used to explain the differences and similarities between samples, and the corresponding load maps could be explained to find out what caused the similarities between samples (Xiao et al., 2015). Therefore, PLSR analysis is more accurate and had great potential in quality control applications. Overall, the flavour fingerprint and E-nose data combined with chemometric analysis may be an efficient method to investigate differential flavour compounds among various samples.

## *4.6. Conclusions*

The formation pathway of flavour compounds in stewed meat, the factors that affect the flavour of meat products, and the extraction and analysis of volatile flavour components were systematically discussed. Flavour is an important edible quality of stewed meat, the flavour components are complex and the formation pathways are diverse, and its formation mechanism needs to be further studied. There are many factors affecting the flavour of stewed meat. To obtain a better flavour, the raw meat and processing technology should be reasonably controlled according to the characteristics of the product. Appropriate extraction methods should be used according to the purpose of analysis. In this study, the SPME method combine with GC-MS/O and GC × GC-TOFMS were mainly used for the extraction and analysis of volatile flavour components in stewed meat.

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**2**

# Chapter Ⅱ. Characterization of volatile compounds in Chinese stewed pork by solid phase microextraction gas chromatography-mass spectrometry/olfactometry, electronic nose and chemometrics

*In China, the stewed pork product is a part of the Chinese time-honoured and intangible cultural heritage. It also occupies a large share in meat products and is favoured by consumers due to its distinct flavour. Most of the previous studies about the stewed pork focused on the flavour analysis of roast, steamed and cooked meat. However, few studies have been reported on the qualitative and quantitative analysis of the volatile compounds of stewed pork. The aim of this chapter was to study volatile composition of stewed pork from the different brands.*

Han, D., Mi, S., Zhang, C. H., Li, J., Song, H. L., Fauconnier, M. L., & Tyteca, E. (2019). Characterization and Discrimination of Chinese Marinated Pork Hocks by Volatile Compound Profiling Using Solid Phase Microextraction Gas Chromatography-Mass Spectrometry/Olfactometry, Electronic Nose and Chemometrics. Molecules, 24(7), 1385.

**Abstract:** The primary aim of this study was to investigate volatile constituents for the differentiation of Chinese stewed pork from four local brands, Dahongmen (DHM), Daoxiangcun (DXC), Henghuitong (HHT) and Tianfuhao (TFH). To this end the volatile constituents were evaluated by gas chromatography-mass spectrometry/olfactometry (GC-MS/O), electronic nose (E-nose) and chemometrics. A total of 62 volatile compounds were identified and quantified in all pork samples, and 24 of them were considered as odour-active compounds because their odour activity values (OAVs) were greater than 1. Hexanal (OAV at 3.6–20.3), octanal (OAV at 30.3–47.5), nonanal (OAV at 68.6–166.3), 1,8-cineole (OAV at 36.4–133.3), anethole (OAV at 5.9–28.3) and 2-pentylfuran (OAV at 3.5–29.7) were the key odour-active compounds contributing to the integral flavour of the stewed pork. According to principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) of GC-MS/O and E-nose data, the results showed that the stewed pork were clearly separated into three groups: DHM, HHT, and DXC-TFH. Nine odour-active compounds, heptanal, nonanal, 3-carene, d-limonene, β-phellandrene, *p*-cymene, eugenol, 2-ethylfuran and 2-pentylfuran, were determined to represent potential flavour markers for the discrimination of stewed pork.

**Keywords:** characterization; discrimination; Chinese stewed pork; odour activity value; key odour-active compounds; potential flavour markers

# 1. Introduction

The preparation of stewed pork in China can be dated back to thousands of years ago. The traditional stewing procedure is as follows: the fresh meat is immersed in marinade for a certain period of time after meat is cooked in liquid at 98 ± 2 °C for 1 h or longer (Wei et al., 2017). Stewed pork, a part of the Chinese time-honored and intangible cultural heritage, is appreciated by Chinese consumers due to its distinct sensory characteristics, such as tender texture, bright color and rich flavor. Among all the sensory attributes, flavor has been rated as one of the most important attributes for this type of products. To date, more than 1000 odorous compounds have been identified in various meat products, including aldehydes, ketones, alcohols, esters, heterocyclic compounds and sulfur-containing compounds (Shahidi, 1998).The comparison of volatiles in the marinated drumsticks using the traditional and quantitative marinated methods found 44 and 60 volatile flavor compounds present in these two types of products, respectively, which showed that the processing method can affect the flavor composition of marinated products greatly (Li et al., 2016). A total of 149 volatile compounds that have been identified in dry-cured hams exposed to different processing methods, and most abundance volatiles in the ham samples were aldehydes (Petričević, Radovčić, Lukić, Listeš, & Medić, 2018). Volatile flavor constituents in roasted pork from miniature pigs were studied, in which aldehydes are believed to play an important role in the flavor composition (Xie, Sun, Zheng, & Wang, 2008). All of these studies above mainly focused on the qualitative and quantitative analysis of the volatile compounds from the different meat products, such as dry-cured hams (Petričević et al., 2018; Wang et al., 2018), roasted pork (Xie et al., 2008) and braised chicken (Duan et al., 2015; Li et al., 2016). However, few studies have been reported on the flavor profiling of Chinese stewed pork.

Solid phase microextraction and gas chromatography-mass spectrometry (SPME-GC-MS) have been applied as a prevalent analytical technique for food aroma analysis due to its ability to separate and identify volatile compounds (Chen, Song, Bi, Meng, & Wu, 2018; Feng et al., 2011; Lorenzo & Fonseca, 2014). However, it is difficult to distinguish the contribution of each single volatile to the integral aroma profile in food. The odor activity value (OAV) is thus introduced. OAV can be calculated based on dividing the concentration of a compound by its mean recognition threshold in the matrix (Gu, Wang, Tao, & Wu, 2013; Zhou, Chong, Ding, Gu, & Liu, 2016). Moreover, one of the weaknesses of the GC-MS method is that it cannot associate compounds with the actual sensory experience. Therefore, linking the sensory experience and chemical compounds identified by gas chromatography-olfactometry (GC-O) was attempted. The most important aroma-active compounds of Beijing roast duck were identified by using aroma extract dilution analysis (AEDA), dynamic headspace dilution analysis, GC-MS/O and OAVs (G. Chen, Song, & Ma, 2009). 2,3-Butanedione, 2,5-dimethylpyrazine, 3-methylbutanal, 2-acetyl-1-pyrroline and 2-acetylthiazole were the key potent contributors in steamed mangrove crab, as determined on the basis of GC-MS/O methods (Yu & Chen, 2010). The volatile compounds were extracted by the simultaneous distillation extraction (SDE) and 31 odor-active compounds were detected and identified in cooked meat of farmed obscure puffer by GC-MS/O (Tao, Wu, Zhou, Gu, & Wu, 2014). The odorous sulfur compounds and three furans were considered as the responsibilities for the “meaty, cooked ham” notes in cooked ham (Thomas, Mercier, Tournayre, Martin, & Berdagué, 2014). These relevant research projects were about the flavor analysis of roast, steamed and cooked meat. However, a comprehensive GC-MS/O method for the identification and quantification of the volatile compounds in the stewed pork has never been reported.

Nowadays, some studies have been reported on characterization and classification of different food using chemometrics analysis. The selected China’s domestic pork were characterized and discriminated using an LC-MS-based lipidomics analysis (Mi, Shang, Li, et al., 2019). The ICP-MS and multivariate analysis of the mineral elementals were applied for the authentication of Taihe black-boned silky fowl muscles (Mi, Shang, Jia, Zhang, & Fan, 2019). The volatile flavor composition can be also used as discriminating parameter for identifying the cooked pork samples from four pig varieties (Yang, Pan, Zhu, & Zou, 2014). However, none of these studies regarding the discrimination of the stewed pork has been used for chemometrics analysis of the volatile compounds. This study was aimed to investigate the volatile profiles of stewed pork from different brands and to determine the key odor-active compounds and potential flavor markers using GC-MS/O. The multivariate statistical techniques, such as PCA and PLS-DA were used to understand the similarities and differences between the stewed pork from different brands.

# 2. Materials and Methods

## *2.1. Materials and chemicals*

The study procedures were approved by the Animal Care and Use Committee of the Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, and were performed in accordance with animal welfare and ethics. Twelve commercial stewed pork were purchased, including the following Chinese brands: Beijing Ershang Group Dahongmen Meat Food Co. Ltd. (labelled as DHM, Beijing, China), Beijing Daoxiangcun Food Co. Ltd. (labelled as DXC, Beijing, China), Beijing Henghuitong Meat Food Co. Ltd. (labelled as HHT, Beijing, China), and Beijing Tianfuhao Food Co. Ltd. (labelled as TFH, Beijing, China). For each brand, three different lot numbers were collected. According to the tracking information, the products were processed using Duroc × (Landrace × Yorkshire) pigs (*n* =12, aged 5–6 months and with body weights of 90–95 kg), and all of the pigs were slaughtered following routine abattoir procedures. After chilling at 2–4 °C for 24 h, the pork was dissected from the individual carcasses. To produce stewed pork, pork was first boiled in water at 100 °C for 10 min to remove blood and then transferred to special marinades for 45 min at 100 °C. The marinade information for each product was summarized in Table 2 - 1. The meat was trimmed to the skin, visible external fat and connective tissues. To minimize the deterioration of volatile components, all samples were cut into small pieces (5 mm × 5 mm × 5 mm) and stored at −18 °C until needed.

**Table 2 - 1:** Ingredient composition of different marinades based on the product labels.

|  |  |
| --- | --- |
| Products | Ingredients |
| DHM | Pork leg meat, salt, soy sauce, white granulated sugar, flavour liquor, soy protein, monosodium glutamate. |
| DXC | Pork leg meat, salt, soy sauce, white granulated sugar, glucose, rice wine, soy protein, spices, monosodium glutamate, pork seasoning. |
| HHT | Pork leg meat, salt, soy sauce, white granulated sugar, glucose, soy protein, spices. |
| TFH | Pork leg meat, salt, sugar, baijiu, spices. |

Saturated alkanes C7–C30 (1000 μg/mL for each component in hexane) and 2-methyl-3-heptanone (99%) were obtained from Sigma-Aldrich (Shanghai, China).

## *2.2. Solid phase microextraction of volatile compounds*

The SPME manual device (Supelco, Inc., Bellefonte, PA, USA) equipped with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre was employed for the extraction of volatile compounds from the stewed pork. The extraction was performed according to the method proposed by Yao et al., (2015) with some modifications. Briefly, 5.0 g of meat sample was accurately weighed and transferred to a 40 mL headspace flask. Then, 1 μL of 2-methyl-3-heptanone solution at 0.41 mg/mL (dissolved in hexane) was added to the homogenized meat sample, acting as the internal standard before the SPME processing. The sample was equilibrated at 60 °C for 20 min and extracted with the selected fibre for 40 min at the same temperature. Upon completion, the fibre was inserted into the injection port (250 °C) of the GC instrument to desorb the analysts for 5 min. All samples were extracted in triplicate.

## *2.3. GC-MS/O analysis of volatile compounds*

All volatile compounds were analysed by GC-MS using an Agilent 7890A gas chromatograph and an Agilent Model 7000B series mass-selective detector with a quadrupole mass analyser (Agilent Technologies, Inc., Santa Clara, CA, USA). An olfactory detector port (Sniffer 9000; Brechbuhler, Schlieren, Switzerland) was coupled with the GC/MS system. After samples were injected, two types of capillary columns, polar DB-Wax and non-polar DB-5 (30 m × 0.32 mm × 0.25 µm; J & W Scientific, Inc., Folsom, CA, USA), were used to perform the separation. Ultra-high purity helium (≥99.999%) was employed as the carrier gas with a constant flow rate of 1.2 mL/min. The oven programme was as follows: the initial column temperature was maintained at 40 °C for 3 min, then increased to 200 °C at a rate of 5 °C/min, and finally increased to 230 °C (DB-Wax) and 250 °C (DB-5) at 10 °C/min for 3 min. The transfer line temperatures were 240 °C (DB-Wax) and 270 °C (DB-5). The effluent from the capillary column was split between the mass spectrometry detector and the olfactory detector port at a ratio of 5:1 (*v/v*). The working conditions of MS were set as follows: ionization energy at 70 eV; scan range at 50–400 *m/z*; and ion source temperature at 230 °C. For the GC-O analysis, the occurrence time and characteristics were recorded by six experienced panellists during the sniffing test. Humidified air was supplied to the sniff port with a flow of 30 mL/min to avoid dryness of the nasal mucosa.

## *2.4. Identification and quantification of volatile compounds*

The volatile components were identified by comparing their electron ionization (EI) spectra with the database records provided by the National Institute of Standards and Technology (NIST) Mass Spectral Library (Version 2.0, Gaithersburg, Maryland, USA). The qualitative determination of these volatile compounds was further confirmed via matching their linear retention indices (LRIs) and odour descriptions in the literature and online databases (http://www.flavornet.org; http://www.odour.org.uk). The LRI values were computed according to the following equation (Wu, Tao, & Gu, 2013):

|  |  |
| --- | --- |
|  | (1) |

where Rt (i) is the retention time of the individual compound under investigation (i) and Rt (n) and Rt (n + 1) refer to the retention times of *n*-alkanes that elute before and after the target compound (i) for the same chromatographic conditions.

Quantitative analysis of the identified volatile compounds was achieved by adding 2-methyl-3-heptanone (internal standard, IS) to the samples prior to the SPME procedures. The relative concentrations of the volatile constituents were determined by the GC-peak areas of calibration curves and the ratios of the target analysts relative to 2-methyl-3-heptanone. The final results were expressed as µg volatile compounds/kg of the stewed pork. Each value represented the average of triplicate determinations. 2-Methyl-3-heptanone was used as the internal standard without considering the calibration factors; thus, all calibration factors were considered to be 1.00. The equation is written as follows:

|  |  |
| --- | --- |
|  | (2) |

The OAV is known as the ratio of the relative concentration (Ci), which is the value of the identified compounds, to the odour threshold (OT) in water. The OAV can be calculated by the following additional equation:

|  |  |
| --- | --- |
|  | (3) |

Compounds with OAV > 1 were considered as odour-active compounds.

## *2.5. E-nose analysis of stewed pork*

A portable electronic nose (PEN3) with an enrichment and desorption unit (EDU) from Win Muster Airsense Analytics, Inc. (Airsense, Schwerin, Germany), was employed to investigate the odour profiles of pork samples. The PEN3 is composed of a sampling apparatus, a detector unit containing ten metal oxide sensors (Gao et al., 2017), and pattern identification software for data recording and elaboration (Liu et al., 2012). Approximately 1.00 g of the stewed pork was added to a 10 mL glass vial. Filtered and dried air with flow rate of 300 mL/min was employed for the headspace injection. The data acquisition period was 60 s, and an additional 180 s was required for system rebalancing. All samples had three replicates and were measured under the same conditions.

## *2.6. Sensory evaluation of stewed pork*

The panel for sensory evaluation included eight trained panellists (four males and four females, aged 25–35 years) from the Chinese Academy of Agriculture, Beijing. All assessors offered at least one year of experience in the descriptive analysis of marinated meat products.

To train the sensory panel to be familiar with the sensory characteristics of marinated pork hocks, training sessions were conducted for 12 weeks (2 times per week), and each session took approximately 2 h. During the training sessions, the panellists, on the basis of available literature (Braghieri, Piazzolla, Carlucci, Bragaglio, & Napolitano, 2016; Lee, Kwon, Kim, & Kim, 2011), developed and defined the sensory attributes, reference standard samples and their intensities (Table 2 -2). Different stewed pork samples were coded with three-digit randomized numbers and served in random order to prevent bias. The evaluation was performed at room temperature, one time each, and with a 5 min wait between samples. The panellists were asked to evaluate six sensory attributes, namely, fatty odour, meaty odour, caramel odour, soy sauce odour, fruity odour and roasted odour. The intensities of six descriptive sensory attributes were evaluated using a 10 cm unstructured line (Penaranda et al., 2017) ranging from “not perceivable” to “strongly perceivable.” The data were presented as the mean values of scores of each odour note and plotted in the radar charts.

**Table 2 - 2:** Definitions of odour attributes and reference standards.

|  |  |  |
| --- | --- | --- |
| Odour Attributes | Definitions | References (Intensity) |
| Fatty | The smell associated with lard oil | Lard oil at 25 °C (6.0) |
| Meaty | The smell associated with cooked pork | 20.0 g of defatted pork in 60.0 mL of water was boiled for 1 h (8.0) |
| Soy sauce | The smell associated with soy sauce | 3.0 g of soy sauce in 50.0 mL of water (7.0) |
| Fruity | The smell associated with fresh fruit | Newly cut orange peel or apple peel (8.0) |
| Caramel | The smell associated with burning white sugar | 5.0 g of burning white sugar in 50.0 mL water (6.0) |
| Roasted | The smell associated with roasted pork | 1.0 kg pork was roasted by charcoal fires for 1 h (6.0) |

## *2.7. Statistical analysis*

Contents of volatile compounds were presented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan’s multiple range tests were carried out by using SPSS software (v. 19.0, SPSS, Inc., Chicago, IL, USA). The significance level was set at *P* < 0.05. PCA and PLS-DA were performed based on the mean OAV of odour-active compounds (OAV > 1) using the software XLSTAT (2016) from Addinsoft (Barcelona, Spain). The odour-active compounds with variable importance, indicated by the projection (VIP) score of > 1 in the PLS-DA analysis and p-value of < 0.05, were considered as significant differences among all stewed pork. Likewise, PCA and PLS-DA of E-nose data were also conducted with XLSTAT (Addinsoft Inc, Longlsland, NY, USA, 2016). All experiments were performed in triplicate.

# 3. Results and Discussion

## *3.1. Volatiles profile of stewed pork characterized by GC-MS/O*

### 3.1.1. Volatile composition of stewed pork

The odor descriptions, thresholds and relative concentrations of the volatile compounds identified in the stewed pork are presented in Table 2.3. A total of 62 volatile compounds were detected in the stewed pork, and there were 35, 37, 33 and 25 volatile compounds in DHM, DXC, HHT and TFH, respectively. These compounds can be further categorized into 10 major groups, as follows: 13 aldehydes, nine alcohols, five ketones, three esters, 14 hydrocarbons, two ethers, two phenols, seven furans, three N-containing compounds and four S-containing compounds. Total estimated concentrations of volatile compounds in DHM, DXC, HHT and TFH were 1224.2 μg·kg−1, 1073.4 μg·kg−1, 1791.8 μg·kg−1 and 822.7 μg·kg−1, respectively. TFH contained the least number and lowest levels of volatile constituents. Conversely, the greatest number and highest levels of volatile compositions were detected in DXC and HHT, respectively.

The majority of aldehydes significantly contributed to the flavor profiles of various food matrices due to their low odor thresholds (Giri, Osako, & Ohshima, 2010; Gu et al., 2013). Aldehydes are mainly generated from two pathways, i.e., lipid oxidation and Strecker degradation of amino acids (Li et al., 2016; Xie et al., 2008). In this study, the 2,4-decadienal isomer with fatty and deep-fried odor had the lowest threshold of 0.07 μg·kg−1 in DHM. However, this compound was not observed in the other three pork samples. Benet et al., (2016) reported that the 2,4-decadienal isomer was considered as an oxidation product of linoleic acid, which was the main polyunsaturated fatty acid found in cooked cured pork ham. Nonanal with a citrus and fatty odor was identified and had the highest concentration in all samples. This aldehyde was generated from lipid oxidative degradation (Wang et al., 2018). Additionally, the remaining four aldehyde compounds, including hexanal, octanal and benzaldehyde, were also detected in all samples. Among these compounds, benzaldehyde (with a bitter almond smell) has the larger odor threshold, which demonstrates the lesser contributions to the aroma profiles of the stewed pork. Note that the aroma profile of DXC was characterized by the presence of 2-methylbutanal and 3-methylbutanal with a nutty odor, which were not detected in the other three samples. Gu et al., (2013) reported that 2-methylbutanal was known to be a Strecker reaction product of isoleucine in steamed Chinese mitten crab. (Liu et al., 2015) found that 3-methylbutanal was formed in salty boiled duck in water, and the formation may be associated with leucine.

Tanchotikul & Hsieh, (1991) reported that alcohols were one of the key flavor compounds in steamed Rangia clam, which could be associated with the decomposition of hydroperoxides of fatty acids or the reduction of aldehydes. Regarding the relative concentrations of alcohols detected in the stewed pork, 1,8-cineole, linalool and terpinen-4-ol were detected in all samples. As shown in Table 1, the relative concentration of 1,8-cineole with the minimum threshold was significantly higher than all other listed alcohol compounds, which indicated its greatest contribution to the complete flavor profile of stewed pork samples. In addition, linalool and 1-octen-3-ol were known to be the most important aroma-active alcohols and have been found in the essential oil (Sánchez-Peña, Luna, García-González, & Aparicio, 2005) and fish products (Zhou et al., 2016), respectively. The β-oxidation of linoleic acid has been considered as the main pathway to form 1-octen-3-ol in dry cured loin (Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004). The odor threshold of 1-octen-3-ol (2 μg·kg−1) was three times lower than that of linalool (6 μg·kg−1); therefore, a higher OAV was achieved by 1-octen-3-ol in DHM, HHT and TFH compared with linalool. In contrast to the above discussed volatile alcohols, terpinen-4-ol, α-terpineol and 2-phenylethanol had very high thresholds, which indicated that they were not the main flavor substances but exerted a synergistic influence on the total flavor.

As for ether compounds, estragole and anethol were identified in all stewed pork (Table 2 - 3). The relative concentrations of estragole and anethol in DHM and TFH were significantly higher than in DXC and HHT (*P < 0.05*), which could be explained by the greater quantities of herbs and spices used in the processing of DHM and TFH. Yao et al., (2015) pointed out that both compounds are the main components in aniseed plants.

**Table 2 - 3:** Odour descriptions, odour thresholds and relative concentrations of volatile compounds in stewed pork by GC-MS/O.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Code | Compounds | Formula | DB-wax e | DB-5 f | Identification | g Odour descriptions | h Odour threshold (μg·kg−1) | Relative concentration (μg·kg−1) | | | |
| DHM | DXC | HHT | TFH |
|  | Aldehydes (13) |  |  |  |  |  |  | 372.0 ± 9.2a | 322.1 ± 18.7a | 142.5 ± 10.1b | 377.0 ± 3.8a |
| 1 | 2-Methylbutanal | C5H10O | 902 | 680 | MS, LRI, O | Nutty | 1 | j N.D. | 10.0 ± 1.2 | N.D. | N.D. |
| 2 | 3-Methylbutanal | C5H10O | 906 | i N.A. | MS, LRI, O | Almond, nutty | 1.1 | N.D. | 11.7 ± 1.8 | N.D. | N.D. |
| 3 | Hexanal | C6H12O | 1073 | 800 | MS, LRI, O | Green, grass, | 4 | 81.0 ± 3.5a | 14.4 ± 1.6d | 46.9 ± 4.6b | 35.6 ± 1.2c |
| 4 | Heptanal | C7H14O | 1179 | 901 | MS, LRI, O | Fatty, oily | 3 | 23.9 ± 1.5a | 25.5 ± 3.1a | N.D. | 16.4 ± 2.3b |
| 5 | Octanal | C8H16O | 1284 | 1003 | MS, LRI, O | Orange peel | 0.7 | 33.2 ± 2.3a | 21.2 ± 1.8c | 25.1 ± 2.2b | 31.9 ± 0.2a |
| 6 | Nonanal | C9H18O | 1389 | 1104 | MS, LRI, O | Citrus, fatty | 1 | 166.6 ± 4.6a | 122.4 ± 9.5c | 68.6 ± 4.9d | 135.9 ± 3.2b |
| 7 | 2-Octenal isomer | C8H14O | 1426 | N.A. | MS, LRI, O | Fatty, green | 3 | 3.7 ± 1.1 | N.D. | N.D. | N.D. |
| 8 | Benzaldehyde | C7H6O | 1520 | 961 | MS, LRI, O | Bitter almond | 350 | 29.2 ± 1.8c | 102.9 ± 1.3b | N.D. | 156.2 ± 1.7a |
| 9 | 2-Nonenal isomer | C9H16O | 1533 | N.A. | MS, LRI, O | Fatty, cucumber | 0.19 | 4.2 ± 1.1 | N.D. | N.D. | N.D. |
| 10 | Anisaldehyde | C8H8O2 | 1683 | N.A. | MS, LRI, O | Mint, sweet | 27 | 13.8 ± 2.1a | 12.7 ± 2.0a | N.D. | N.D. |
| 11 | 2,4-Decadienal isomer | C10H16O | 1719 | N.A. | MS, LRI, O | Fatty, deep-fried | 0.07 | 15.8 ± 1.2 | N.D. | N.D. | N.D. |
| 12 | Pentadecanal | C15H30O | N.A. | 1712 | MS, LRI | Fresh | N.A. | N.D. | 0.2 ± 0.1 | N.D. | N.D. |
| 13 | Hexadecanal | C16H30O | N.A. | 1793 | MS, LRI | Cardboard | N.A. | 0.5 ± 0.1c | 1.1 ± 0.1b | 1.8 ± 0.3a | 1.1 ± 0.3b |
|  | Alcohols (9) |  |  |  |  |  |  | 127.4 ± 0.6c | 107.5 ± 7.4d | 249.8 ± 13.4a | 202.8 ± 15.2b |
| 14 | 1,8-Cineole | C10H18O | 1204 | 1034 | MS, LRI, O | Mint, sweet | 1 | 38.1 ± 1.3c | 36.4 ± 0.3c | 113.3 ± 12.1b | 133.3 ± 13.4a |
| 15 | 1-Hexanol | C6H14O | 1349 | N.A. | MS, LRI | Flower, green | 2500 | N.D. | N.D. | 18.9 ± 2.6 | N.D. |
| 16 | 1-Octen-3-ol | C8H16O | 1445 | 981 | MS, LRI, O | Mushroom | 2 | 10.6 ± 0.3b | N.D. | 16.6 ± 1.8a | 14.3 ± 1.3a |
| 17 | *cis*-4-Thujanol | C10H18O | 1462 | 1071 | MS, LRI | Balsamic | N.A. | 15.7 ± 0.2 | N.D. | N.D. | N.D. |
| 18 | Linalool | C10H18O | 1541 | 1101 | MS, LRI, O | Aniseed, citrus | 6 | 13.4 ± 0.7b | 9.7 ± 1.8c | 18.9 ± 1.5a | 12.7 ± 1.3b |
| 19 | 1-Octanol | C8H18O | 1553 | N.A. | MS, LRI, O | Herbal, green | 110 | N.D. | N.D. | 9.2 ± 0.7 | N.D. |
| 20 | Terpinen-4-ol | C10H18O | 1601 | 1183 | MS, LRI, O | Musty, terpene | 340 | 38.4 ± 0.6b | 31.4 ± 4.3bc | 72.9 ± 3.9a | 34.1 ± 1.0c |
| 21 | α-Terpineol | C10H18O | 1695 | 1196 | MS, LRI, O | Oil, anise, mint | 350 | 11.1 ± 0a | 5.7 ± 0.2c | N.D. | 8.4 ± 0.8b |
| 22 | 2-Phenylethanol | C8H10O | 1908 | N.A. | MS, LRI | Perfumy, rose | 1100 | N.D. | 24.3 ± 1.2 | N.D. | N.D. |
|  | Ketones (5) |  |  |  |  |  |  | 0.6 ± 0.1d | 9.6 ± 1.6c | 40.7 ± 4.5a | 15.0 ± 1.2b |
| 23 | 2-Butanone | C4H8O | 885 | N.A. | MS, LRI | Ethereal, cheesy | 35400 | N.D. | N.D. | N.D. | 11.7 ± 0.9 |
| 24 | 2-Heptanone | C7H14O | 1179 | 890 | MS, LRI, O | Blue cheese | 140 | N.D. | N.D. | 34.9 ± 3.6 | N.D. |
| 25 | 6-Methyl-5-hepten-2-one | C8H14O | N.A. | 987 | MS, LRI, O | Pepper, rubber | 50 | 0.6 ± 0.1 | N.D. | N.D. | N.D. |
| 26 | 2-Nonanone | C9H18O | 1386 | N.A. | MS, LRI, O | Hot milk, green | 200 | N.D. | N.D. | 5.9 ± 1.0 | N.D. |
| 27 | Piperitone | C10H16O | 1583 | 1260 | MS, LRI, O | Mint, fresh | N.A. | N.D. | 9.6 ± 1.6 | N.D. | 3.4 ± 0.2 |
|  | Esters (3) |  |  |  |  |  |  | 47.6 ± 0.8b | 92.9 ± 4.7a | 16.2 ± 3.9c | 10.7 ± 1.6c |
| 28 | Ethyl acetate | C4H8O2 | 869 | N.A. | MS, LRI | Fruity, sweet | 5 | 15.5 ± 0.2b | 92.9 ± 4.7a | N.D. | 10.7 ± 1.6c |
| 29 | Ethyl hexanoate | C8H16O2 | 1229 | N.A. | MS, LRI, O | Apple, sweet | 30 | 32.1 ± 0.6 | N.D. | N.D. | N.D. |
| 30 | Terpinyl acetate | C12H20O2 | 1695 | 1354 | MS, LRI | Fruity, mint | N.A. | N.D. | N.D. | 16.2 ± 3.9 | N.D. |
|  | Hydrocarbons (14) |  |  |  |  |  |  | 34.8 ± 1.8c | 99.6 ± 3.2b | 591.0 ± 18.4a | 46.9 ± 4.7c |
| 31 | Decane | C10H22 | 993 | 1206 | MS, LRI | Irritant | 741 | N.D. | N.D. | 20.8 ± 2.4 | N.D. |
| 32 | Toluene | C7H8 | 1031 | 765 | MS, LRI, O | Rubber, pungent | 1550 | 10.0 ± 1.0c | 16.7 ± 1.4b | N.D. | 23.9 ± 2.8a |
| 33 | β-Pinene | C10H16 | N.A. | 975 | MS, LRI | Benzene-like | 140 | N.D. | N.D. | 22.6 ± 0.9 | N.D. |
| 34 | Ethylbenzene | C8H10 | 1122 | N.A. | MS, LRI, O | Ethereal, floral | 2205.3 | N.D. | N.D. | 5.0 ± 1.5 | N.D. |
| 35 | *p*-Xylene | C8H10 | 1133 | 870 | MS, LRI, | N.A. | 490 | N.D. | 6.6 ± 0.2b | 9.5 ± 0.4a | N.D. |
| 36 | 3-Carene | C10H16 | 1144 | 1012 | MS, LRI, O | Resin, lemon | 0.4 | N.D. | N.D. | 45.2 ± 3.3 | N.D. |
| 37 | α-Terpinene | C10H16 | 1172 | 1018 | MS, LRI | N.A. | 200 | 11.3 ± 0.4b | 7.6 ± 1.7c | 16.8 ± 3.7a | N.D. |
| 38 | Dodecane | C12H26 | 1076 | 1200 | MS, LRI | Irritant | 2040 | N.D. | 17.7 ± 0.6a | N.D. | 16.4 ± 1.1a |
| 39 | d-Limonene | C10H16 | 1186 | 1031 | MS, LRI, O | Fresh | 10 | N.D. | 16.8 ± 1.9b | 263.9 ± 13.6a | N.D. |
| 40 | β-Phellandrene | C10H16 | 1202 | N.A. | MS, LRI, O | Turpentine, mint | 8 | N.D. | 11.8 ± 2.2b | 95.7 ± 6.4a | N.D. |
| 41 | *trans*-β-Ocimene | C10H16 | 1231 | 1039 | MS, LRI | Sweet, herb | N.A. | N.D. | N.D. | 67.5 ± 4.4 | N.D. |
| 42 | *p*-Cymene | C10H14 | 1265 | 1027 | MS, LRI, O | Fruity, herbal | 13 | 12.3 ± 0.5b | 11.5 ± 1.3b | 36.4 ± 6.1a | 6.6 ± 1.7b |
| 43 | 1,3,5-Trimethylbenzene | C9H12 | 1277 | N.A. | MS, LRI | Peculiar | 229 | N.D. | 8.4 ± 1.8 | N.D. | N.D. |
| 44 | Naphthalene | C10H8 | 1741 | N.A. | MS, LRI, O | Camphoric | 60 | 1.2 ± 0.1c | 4.6 ± 1.0b | 7.6 ± 0.8a | N.D. |
|  | Ethers (2) |  |  |  |  |  |  | 529.3 ± 24.6a | 127.9 ± 3.1c | 398.5 ± 2.3b | 93.9 ± 6.9d |
| 45 | Estragole | C10H12O | 1667 | 1201 | MS, LRI, O | Liquorice-like | 6 | 105.3 ± 1.6a | 5.1 ± 0.4c | 21.8 ± 1.5b | 4.6 ± 0.6c |
| 46 | Anethol | C10H12O | 1824 | 1290 | MS, LRI, O | Aniseed-like | 15 | 424.0 ± 22.9a | 122.8 ± 3.5c | 376.8 ± 3.7b | 89.2 ± 6.7d |
|  | Phenols (2) |  |  |  |  |  |  | 10.6 ± 2.6c | 32.1 ± 1.1b | 117.8 ± 11.3a | N.D. |
| 47 | Eugenol | C10H12O2 | 2164 | 1364 | MS, LRI, O | Spicy, clove | 7.1 | 6.6 ± 1.5c | 32.1 ± 1.1b | 117.8 ± 11.3a | N.D. |
| 48 | Isoeugenol | C10H12O2 | 2255 | N.A. | MS, LRI | Floral, spicy | N.A. | 4.0 ± 1.1 | N.D. | N.D. | N.D. |
|  | Furans (7) |  |  |  |  |  |  | 71.3 ± 2.9d | 269.1 ± 13.3b | 230.0 ± 13.6a | 63.3 ± 2.8c |
| 49 | 2-Methylfuran | C5H6O | 847 | N.A. | MS, LRI | Chocolate | 3500 | N.D. | 21.0 ± 1.4a | N.D. | 12.2 ± 2.5b |
| 50 | 2-Ethylfuran | C6H8O | 952 | N.A. | MS, LRI, O | Rubber, pungent | 2.3 | N.D. | N.D. | 11.9 ± 2.5 | N.D. |
| 51 | 2-Pentylfuran | C9H14O | 1225 | 992 | MS, LRI, O | Fruity, sweet | 6 | 21.0 ± 2.7d | 28.3 ± 5.8c | 178.2 ± 3.7a | 40.2 ± 1.2b |
| 52 | Furfural | C5H4O2 | 1457 | 829 | MS, LRI, O | Almond, sweet | 3000 | 26.6 ± 0.4b | 62.8 ± 3.4a | 16.7 ± 4.4c | 5.1 ± 0.3d |
| 53 | 2-Acetylfuran | C6H6O2 | 1500 | 912 | MS, LRI, O | Sweet, smoky | 80000 | 12.6 ± 0b | 30.7 ± 1.5a | 12.7 ± 2.9b | N.D. |
| 54 | 5-Methylfurfural | C6H6O2 | 1568 | N.A. | MS, LRI, O | Almond, sweet | 1100 | 4.6 ± 0.4b | 92.9 ± 2.5a | N.D. | N.D. |
| 55 | 2-Furanmethanol | C5H6O2 | 1652 | 863 | MS, LRI, O | Sweet, honey | 2000 | 6.5 ± 0.2bc | 33.4 ± 3.7a | 11.5 ± 3.9b | 5.8 ± 0.6c |
|  | *N*-containing compounds (3) |  |  |  |  |  |  | 17.8 ± 1.8a | 8.5 ± 0.4b | N.D. | 5.5 ± 0.3c |
| 56 | 2-Methylpyrazine | C5H6N2 | 1262 | N.A. | MS, LRI, O | Popcorn | 250 | N.D. | N.D. | N.D. | 5.5 ± 0.3 |
| 57 | 1-Vinylimidazole | C5H6N2 | 1263 | N.A. | MS, LRI | N.A. | N.A. | N.D. | 8.5 ± 0.4 | N.D. | N.D. |
| 58 | 2-Acetylpyrazine | C6H6N2O | 1624 | N.A. | MS, LRI, O | Nutty, roast | 62 | 17.8 ± 1.8 | N.D. | N.D. | N.D. |
|  | *S*-containing compounds (4) |  |  |  |  |  |  | 12.9 ± 1.2a | 4.3 ± 1.2c | 4.2 ± 1.0c | 7.5 ± 0.8b |
| 59 | 3-Methylthiophene | C5H6S | 1083 | N.A. | MS, LRI, O | Fatty, wine | 5000 | N.D. | 4.3 ± 1.2b | N.D. | 7.5 ± 0.8a |
| 60 | 2-Methylthiophene | C5H6S | 1075 | N.A. | MS, LRI, O | Gasoline, green | 3000 | N.D. | N.D. | 4.2 ± 1.0 | N.D. |
| 61 | 2-Acetylthiazole | C5H5NOS | 1644 | N.A. | MS, LRI, O | Caramel, sweet | 10 | 10.7 ± 1.5 | N.D. | N.D. | N.D. |
| 62 | 3-(Methylthio)propanol | C4H10OS | 1712 | N.A. | MS, LRI | Sweet, potato | 123 | 2.2 ± 0.8 | N.D. | N.D. | N.D. |
|  | Total |  |  |  |  |  |  | 1224.2 ± 34.7b | 1073.4 ± 35.3c | 1791.8 ± 64.8a | 822.7 ± 26.2d |

Note: Different letters indicate significant differences (*P* < 0.05) among samples. All experiments were conducted for *n* = 3 independent stewed pork. Standard deviations are shown. MS, mass spectrum comparison using NIST libraries; LRI, linear retention index compared with literature value; O, odour description.  
e Odour descriptions were mainly gathered from the following literature and online database: (Buttery et al., 1974; G. Chen et al., 2009; Giri et al., 2010; Gu et al., 2013; C. Yang et al., 2010; Zhou et al., 2016), <http://www.flavornet.org>, http://www.odour.org.uk.  
f Odour thresholds were mainly obtained from the literature and an online database, with water applied as the matrix: (G. Chen et al., 2009; Giri et al., 2010; Gu et al., 2013; J. Liu et al., 2015; Zhou et al., 2016), <http://www.flavornet.org>, <http://www.odour.org.uk>.   
g Linear retention index on DB-wax column.  
h Linear retention index on DB-5 column.  
i N.D., not detectable.  
g N.A., not available.

Another main observation from Table 2 -3 is the relatively high odor threshold of hydrocarbons, which results in low contributions for the majority of the hydrocarbons except ethyl acetate, 3-carene, D-limonene and β-phellandrene. As reported, hydrocarbons can be derived from alkyl radicals via lipid auto-oxidation processes (Fu, Xu, & Wang, 2009). Other types of volatiles including ketones, esters and phenols are also considered as a flavor auxiliary of the stewed pork, although they have relatively high thresholds.

Furans refer to a group of heterocyclic compounds that were structurally characterized with the oxygen atom in the ring. Giri et al., (2010) reported that furans might be derived from the dehydration of carbohydrates or the Amadori rearrangement procedure. Taylor & Mottram, (1990) suggested that the oxidation of fatty acids could be another pathway for the formation of furans. Among six furans listed in Table 2 - 3, 2-pentylfuran with the lower threshold (6.0 μg·kg−1) had the highest relative concentration in all samples, and 2-ethylfuran with the lowest threshold (2.3 μg·kg−1) had the highest relative concentration in only HHT. These two furans were oxidative degradation products of linolenate (Chen et al., 2009; Gu et al., 2013) and were considered as the most important flavor compounds contributing to meat products (Gu et al., 2013).

Several N- and S-containing compounds were detected in the stewed pork. They were mainly derived from the catabolism of proteins, free amino acids and nucleic acids (Chen et al., 2009). The 2-acetylthiazole with roasted and caramel notes was usually considered as an important flavor compound contributing to the flavor of cooked meat (Chen et al., 2009; Machiels, 2003).

### 3.1.2. OAVs of the odour-active compounds

The OAV (Equation (3)) was employed to evaluate the contribution of volatile compounds to the aroma profile of the investigated samples. The results are summarized in Table 2 - 4.

**Table 2 - 4:** OAVs of odour-active compounds in stewed pork.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Code | Compounds | Odour activity values (OAVs) | | | | *P* value | VIP value |
| DHM | DXC | HHT | TFH |
| 1 | 2-Methylbutanal | 0.0b | 10.0 ± 1.2a | 0.0b | 0.0b | 0.000 | 0.827 |
| 2 | 3-Methylbutanal | 0.0b | 10.6 ± 1.6a | 0.0b | 0.0b | 0.000 | 0.824 |
| 3 | Hexanal | 20.3 ± 0.9a | 3.6 ± 0.4d | 11.7 ± 1.2b | 8.9 ± 0.3c | 0.000 | 0.817 |
| 4 | Heptanal | 8.0 ± 0.5a | 8.5 ± 1.0a | 0.0c | 5.5 ± 0.8b | 0.000 | 1.068 |
| 5 | Octanal | 47.5 ± 3.2a | 30.3 ± 2.6c | 35.9 ± 3.1b | 45.6 ± 0.2a | 0.000 | 0.867 |
| 6 | Nonanal | 166.6 ± 4.6a | 122.4 ± 9.5c | 68.6 ± 4.9d | 135.9 ± 3.2b | 0.000 | 1.078 |
| 7 | 2-Octenal isomer | 1.2 ± 0.4a | 0.0b | 0.0b | 0.0b | 0.000 | 0.946 |
| 9 | 2-Nonenal isomer | 22.2 ± 5.7a | 0.0b | 0.0b | 0.0b | 0.000 | 0.956 |
| 11 | 2,4-Decadienal isomer | 225.5 ± 17.7a | 0.0b | 0.0b | 0.0b | 0.000 | 0.980 |
| 14 | 1,8-Cineole | 38.1 ± 1.3c | 36.4 ± 0.3c | 113.3 ± 12.1b | 133.3 ± 13.4a | 0.000 | 0.954 |
| 15 | 1-Octen-3-ol | 5.3 ± 0.2c | 0.0d | 8.3 ± 0.9a | 7.2 ± 0.7b | 0.000 | 0.943 |
| 16 | Linalool | 2.2 ± 0.1b | 1.6 ± 0.3c | 3.1 ± 0.2a | 2.1 ± 0.2b | 0.000 | 0.978 |
| 28 | Ethyl acetate | 3.1 ± 0b | 18.6 ± 0.9a | 0.0d | 2.1 ± 0.3c | 0.000 | 0.892 |
| 29 | Ethyl hexanoate | 1.1 ± 0.0a | 0.0b | 0.0b | 0.0b | 0.000 | 0.983 |
| 36 | 3-Carene | 0.0b | 0.0b | 113.0 ± 8.3a | 0.0b | 0.000 | 1.078 |
| 39 | d-Limonene | 0.0c | 1.7 ± 0.2b | 26.4 ± 1.4a | 0.0c | 0.000 | 1.082 |
| 40 | β-Phellandrene | 0.0c | 1.5 ± 0.3b | 12.0 ± 0.8a | 0.0c | 0.000 | 1.081 |
| 42 | *p*-Cymene | 0.9 ± 0.0b | 0.9 ± 0.1b | 2.8 ± 0.5a | 0.5 ± 0.1b | 0.000 | 1.039 |
| 45 | Estragole | 17.5 ± 0.3a | 0.9 ± 0.1c | 3.6 ± 0.2b | 0.8 ± 0.1c | 0.000 | 0.934 |
| 46 | Anethol | 28.3 ± 1.5a | 8.2 ± 0.2c | 25.1 ± 0.2b | 5.9 ± 0.4d | 0.000 | 0.791 |
| 47 | Eugenol | 0.9 ± 0.2c | 4.5 ± 0.2b | 16.6 ± 1.6a | 0.0d | 0.000 | 1.073 |
| 50 | 2-Ethylfuran | 0.0b | 0.0b | 5.2 ± 1.1a | 0.0b | 0.000 | 1.060 |
| 51 | 2-Pentylfuran | 3.5 ± 0.5d | 4.7 ± 0.1c | 29.7 ± 0.6a | 6.7 ± 0.2b | 0.000 | 1.085 |
| 61 | 2-Acetylthiazole | 1.1 ± 0.2a | 0.0b | 0.0b | 0.0b | 0.000 | 0.974 |

Different letters refer to statistically significant differences (*P* < 0.05). Values represent means and standard deviation (*n* = 3).

Twenty-four volatile compounds with OAVs > 1 were selected as odour-active compounds contributing primarily to the total flavour of the stewed pork. A point worth emphasizing is that six odour-active components with relatively high OAVs were simultaneously detected in four samples: hexanal (OAV at 3.6–20.3), octanal (OAV at 30.3–47.5), nonanal (OAV at 68.6–166.3), 1,8-cineole (OAV at 36.4–133.3), anethol (OAV at 5.9–28.3) and 2-pentylfuran (OAV at 3.5–29.7). 1,8-Cineole and linalool constituted a large portion of the specific aroma of Chinese stewed meat products and could have originated from the Chinese traditional spices. Hexanal, octanal, nonanal and 2-pentylfuran with fat and meat flavours were generated from the boiling procedure of the stewed meat products. These components were defined as the key odour-active compounds due to their significant contributions (*P* < 0.05) to the integral flavour. Considering the VIP scores and *p*-values of odour-active compounds, nine of the compounds had a VIP score > 1 and *p*-value < 0.05 and were considered as potential discriminatory markers for the differentiation of stewed pork. These odour-active compounds included heptanal, nonanal, 3-carene, d-limonene, β-phellandrene, *p*-cymene, eugenol, 2-ethylfuran and 2-pentylfuran.

## *3.2. Discrimination of stewed pork by GC-MS/O*

To visualize a total picture of the distributions of 24 odour-active compounds (OAV > 1) in all samples, PCA was applied (Figure. 2 - 1). The two principal axes accounted for 84.69% of the entire variations of the four pork samples; the two PCA components, PC1 and PC2, explained 48.56% and 36.13% of the variation, respectively. As shown in Figure. 2 - 1A, it can be found that the sample dots of the stewed pork were well separated. PC1 clearly distinguished DHM and HHT. DHM was in the positive side of PC1, while HHT appeared in the negative side, indicating that there were obvious differences of flavour features. Both DXC and TFH were located in the lower left quadrant of PC and close to each other, which meant that they have similar flavour. Hence, the four different stewed pork were divided into three groups, i.e., group I: DHM, group II: HHT, and group III: DXC-TFH.

**B**

HHT

TFH

DXC

DHM

**Figure 2 - 1:** (**A**) PCA for odour-active compounds of the four stewed pork. The blue dots represent the samples from stewed pork, and the red dots represent odour-active compounds. (**B**) PLS-DA score plot from different marinated samples (R2X = 0.978, R2Y = 0.997, Q2 = 0.994). The red dots represent DHM, the yellow dots represent HHT, the blue dots represent TFH, and the green dots represent DXC.

Moreover, the major odor-active compounds contributing to DHM were hexanal (green, grass), 2,4-decadienal isomer (fatty, deep-fried), 2-acetylthiazole (caramel, sweaty), ethyl hexanoate (fruity, sweet), 2-nonenal isomer (fatty, cucumber), 2-octenal isomer (fatty, green), estragole (aniseed-like) and octanal (orange peel, fatty). As seen from Table 2 - 4, the OAV values of these aldehyde compounds in DHM were significantly higher than those in the other samples (*p < 0.05*). As had been reported on all types of foodstuff (Wang et al., 2018; Zhang et al., 2018; Zhao et al., 2017), the eight compounds above have been widely studied with respect to their sources and contributions to food aroma (Liu, He, & Song, 2018; Lou; Petričević et al., 2018). HHT was in the first quadrant and highly associated with four hydrocarbons (*p*-cymene, 3-carene, D-limonene and β-phellandrene) and two furans (2-ethylfuran and 2-pentylfuran). Among them, the hydrocarbon compounds could originate from the animal feeds (Sánchez-Peña et al., 2005) and Chinese traditional spices (Li et al., 2016). 2-ethylfuran, 2-pentylfuran and eugenol could have been found in the cooked meat products (Roldan, Ruiz, Del Pulgar, Perez-Palacios, & Antequera, 2015; Yang et al., 2010) and the traditional salted vegetables (Buttery et al., 1974). Only three volatile components were related to DXC and TFH, including ethyl acetate, 2-methylbutanal and 3-methylbutanal. As discussed previously, the formation of 2-methylbutanal and 3-methylbutanal can be attributed to the amino acid Strecker reaction (Giri et al., 2010; Wang et al., 2018).

In addition to PCA analysis, PLS-DA was applied for the discrimination of all stewed pork. Figure. 2 - 1B illustrated the PLS-DA score of the stewed samples. HHT was located on the negative side of axis 1, DHM was found on the positive side of axis 2, and TFH and DXC were clustered on the negative side of axis 2. Hence, there were three separate groups (HHT, DHM and DXC-TFH) for stewed pork. It could also be concluded that the flavor of DXC and TFH was similar, and they were different from HHT and DHM.

## *3.3. Volatile profile of stewed pork characterized by E-nose*

The E-nose responses to the stewed pork were shown in Figure. 2 - 2, where G/G0 was considered as the response value.

G/G0

Time (s)

**Figure 2 - 2:** Response curves of E-nose sensors (S1–S10) to DHM, DXC, HHT and TFH.

Each curve represented the response values of the corresponding sensors varying with time. The variation trends of signals in all samples showed similar changes. The response values of S2 (broad range of nitrous oxides), S7 (terpenes and sulphur-containing organic compounds), and S9 (aromatics and organic sulfides) obviously increased, whereas those of S1 (aromatic compounds) and S8 (broad alcohols) gradually increase to a slight extent. Meanwhile, the signals of S3 (aroma components, ammonia) and S5 (alkane, aromatics, and small polar compounds) showed insignificant changes. The values of G/G0 of S4, S6 and S10 were below one. These sensors were mainly sensitive to hydrogen, broad methane and aliphatic methane, respectively. Compared with all stewed samples, it was found that the signal intensities of DHM showed apparent differences. This result suggested different flavour characteristics. According to the sensor signals, it was difficult to distinguish the stewed samples. Therefore, further analysis was applied by PCA and PLS-DA in following study.

## *3.4. Discrimination of stewed pork by E-nose*

E-nose analysis was performed to evaluate the differences in the aroma profiles of four brands of the stewed pork. The PCA score and load plots of the data obtained by E-nose are shown in Figure. 2 - 3A. The plot consists of two axes, PC1 and PC2. PC1 explains 68.75%, whereas PC2 explains 22.52% of the sample variance. The total cumulative contribution rate of PC1 and PC2 exceeded 85.0% (Yang et al., 2016) which indicated that the maximum variation of the aroma compositions of the stewed pork was well explained by PCA analysis (Liu et al., 2018).

Apart from PCA analysis, PLS-DA was used to distinguish all stewed pork. From Figure. 2 - 3B, the data points of DXC and TFH were closely allocated in the second quadrant; the data points of DHM were on the positive side in axis 1 and those of HHT were in the third quadrant and on the negative side of axis 1. This means that DXC and TFH share similar flavour profiles, although there are numerous different compounds (aldehydes and hydrocarbons) for DHM and HHT.

**A**

**B**

**Figure 2 - 3: (A)** Biplot (score plots and load plots) for PCA based on sensor response data. The blue dots represent the samples from stewed pork, and the red dots represent different sensors. (**B**) PLS-DA of E-nose response data for different marinated samples (R2X = 0.997, R2Y = 0.829, Q2 = 0.407). The blue dots represent DHM, the red dots represent HHT, the purple dots represent TFH, and the green dots represent DXC.

## *3.5. Sensory analysis of stewed pork*

The sensory descriptive analysis of the flavour profiles of each sample is shown in Figure. 2 - 4. The aroma of the stewed pork was described as having a meaty odour, roasted odour, fruity odour, soy sauce odour, fatty odour and caramel notes. The intensities of fatty odour, meaty odour, roasted odour and soy sauce odour in DHM were higher than those in other samples, which could be mainly attributed to aldehydes and *N*- and *S*-containing compounds (e.g., nonanal, 2-nonenal isomer, 2,4-decadienal isomer, 2-acetylthiazole and 3-(methylthio)propanol). It was shown that this result was consistent with Table 1. These volatile compounds were detected in the pork broth of black pig (Zhao et al., 2017) and Chinese-type soy sauce (Gao et al., 2017). The strong fruity smell was presented in TFH because of the high levels of contributions of total alcohols (24.7%). Wang et al., (2018) reported that alcohols have pleasant fruity and floral odours in Chinese dry-cured ham. In addition, caramel notes of HHT had the highest scores in all samples, indicating that HHT was highly associated with furans, such as 2-ethylfuran and 2-pentylfuran.

**Figure 2 - 4:** Radar charts of sensory analysis of the stewed pork.

# 4. Conclusions

In this study, 62 volatile compounds were identified and quantified in stewed pork using SPME-GC-MS/O. These compounds can be divided into ten categories, including aldehydes, alcohols, ketones, esters, hydrocarbons, ethers, phenols, furans, *N*-containing compounds and *S*-containing compounds. The key odour-active volatiles of all evaluated samples were determined as hexanal, octanal, nonanal, 1,8-cineole, anethol and 2-pentylfuran due to their relatively higher OAVs compared with other compounds. Moreover, through multivariate statistics including PCA and PLS-DA analysis, the stewed pork of four brands could be classified into three separate groups (DHM, HHT and DXC-TFH). Nine odour-active compounds were determined as potential flavour makers for the differentiation of stewed pork.

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**3**

Chapter Ⅲ. Characterization and differentiation of boiled pork from Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire) pigs by volatiles profiling and chemometrics analysis

*The flavor composition in stewed pork were comprehensively analyzed in chapter 2. The formation of many flavor compounds is affected by many factors, such as pork variety, spices, processing methods, etc. Among them, the pork variety plays an important role in stewed pork. In recent years, many reports are about the volatile compounds of pork from different processing technologies. However, the volatile profiles in different varieties of processed pork products are still lacking. This chapter characterize the volatile compounds of boiled pork form different varieties. In addition, it provided a promising approach of GC-MS/O combined with E-nose to differentiate different varieties of boiled pork.*

Han, D., Zhang, C. H., Fauconnier, M. L., & Mi, S. (2020). Characterization and differentiation of boiled pork from Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire) pigs by volatiles profiling and chemometrics analysis. *Food Research International*, *130*, 108910.

**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. The study indicated that the influence of different pig breeds on flavour is greater than from different pig parts for boiled pork by E-nose analysis. These analyses provided a reliable method to determine and distinguish the volatile profiles of different varieties of samples using GC-MS/O and E-nose.

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

**Chemical compounds studied in this article:**

Hexanal (PubChem CID: 6184)

Nonanal (PubChem CID: 31289)

1-Octen-3-ol (PubChem CID: 18827)

Dimethyl disulphide (PubChem CID: 12232)

Heptanal (PubChem CID: 8130)

2-Pentylfuran (PubChem CID: 19602)

2-Ethylfuran (PubChem CID: 18554)

(*E,E*)-2,4-Decadienal (PubChem CID: 5283349)

2-Acetylthiazole (PubChem CID: 520108)

# 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, Bjergegaard, & 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 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 multivarise statistics is a promising approach for the differentiatation 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 odour-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.

# 2. Materials and methods

## *2.1.* *Materials and chemicals*

A total of 18 pigs from three breeds, including Tibetan pigs (n = 6, aged 5-6 months), Sanmenxia pigs (n = 6, aged 5-6 months) and Duroc × (Landrac × Yorkshire) (n = 6, aged 5-6 months) were studied. Tibetan pigs (TB) was provided by Tibet Woye Tibetan Pig Development Co. Ltd. (Nyingchi, Tibet Autonomous Region, China). Sanmenxia pigs (SMX) and Duroc × (Landrace × Yorkshire) pigs (DLY) were obtained from Chuying Agro-Pastoral Group Co. Ltd. (Zhengzhou, Henan Province, China). All the pigs were reared under the same conditions and provided with the same feed. They were slaughtered following the same commercial procedures in the nearby abattoir. After cooling at 0-4 °C for 24 h, the fore leg muscles and hind leg muscles of all of the pigs were dissected from the carcasses. Tibetan, SMX and DLY pork samples (n = 6 for each breed) from two different muscle were collected, and the same muscle gathered from two individual pigs of the same breed was combined as one sample (n = 3 for each muscle) for volatiles analysis. All pork samples were placed into ice-boxes and sent to the laboratory of Chinese Academy of Agricultural Sciences, Beijing. The study procedures were approved by the Animal Care and Use Committee of the Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, and performed in accordance with animal welfare and ethics.

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

## *2.2. Boiled pork muscles pre-treatment*

The skin, visible fat and connective tissues were removed from the pork of TB, SMX and DLY. Approximately 200 g of meat supplemented with 1.0% sodium chloride (based on the raw meat weight) and 150% (*w/w*) tap water were boiled in a low-density polyethylene bag. The pork samples were first heated from room temperature (22.3 ± 0.5 °C) to a core temperature (80.0 ± 0.5 °C), then held for 30 min. The boiled pork was cut into 1.0 × 1.0 × 1.0 cm3 cubes, ground with a pulveriser in liquid nitrogen and stored in a frozen state (-18 °C) until use.

## *2.3.* *Solid-phase micro-extraction (SPME) of volatile compounds*

The extraction method was modified from a previous study (Wang, Song, Zhang, Tang, & Yu, 2016). The volatile compounds of boiled pork were extracted using a manual SPME equipped with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, Inc., Bellefonte, PA, USA). The pork sample was precisely weighed—5.00 g—and transferred to a 40 ml vial. Thereafter, 2-methyl-3-heptanone was dissolved in hexane as an internal standard solution to a final concentration of 0.41 mg/ml, 1μl of this solution was added and the vial was tightly capped with Teflon/silicon septum. The vial was equilibrated at 60 °C for 20 min in a water bath. The selected fibre was exposed to the headspace of the samples to absorb the volatile compounds for 40 min at 60 °C. Upon completion, the fibre was inserted into the injection port (250 °C) of the GC instrument to desorb the analyses for 5 min. All samples were extracted in triplicate.

## *2.4.* *GC-MS/O analysis*

The method was performed according to the method of Liu, He, and Song (2018) with minor modifications. Analyses of volatile compounds were performed out on an Agilent 7890A gas chromatograph coupled with an Agilent Model 7000B series mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA). The GC-MS system was equipped with an olfactory detector port (Sniffer 9000; Brechbuhler, Schlieren, Switzerland). The volatiles were separated on polar DB-wax and non-polar DB-5 capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness; J & W Scientific, Inc., Folsom, CA, USA). Ultra-high purity helium (≥99.999%) was used as the carrier gas and the constant flow rate was 1.2 ml/min. Temperature programme began with isothermal heating at 40 °C for 3 min, then rising to 200 °C at a rate of 5 °C/min, followed by another increase to 230 °C (DB-wax) and 250 °C (DB-5) at 10 °C/min. Final temperature was held for 3 min. The transfer line temperatures were maintained at 240 °C (DB-wax) and 270°C (DB-5). The effluent from the capillary column was split 5:1 (*v/v*) between the mass spectrometry detector and the olfactory detector port. Electro-impact mass spectra were generated at 70 eV with an *m/z* scan range from 50 to 400 amu. The ion source temperature was 230 °C. A panel that contains eight trained staff was utilized for the sniffing test on the GC-O. Humidified air was supplied to the sniff port with a flow of 30 ml/min to avoid dryness of the nasal mucosa.

## *2.5.* *Identification and quantification of volatile compounds*

The volatile components were identified by comparing their electron ionization (EI) spectra with the database records provided by the National Institute of Standards and Technology (NIST) Mass Spectral Library (Version 2.0), GC retention indices (RIs) and odour descriptions described in the literature and in online databases (http://www.flavornet.org; http://www.odour.org.uk).

Quantitative analysis of the volatile compounds was performed using a calibration method with an internal standard (Zhou, Chong, Ding, Gu, & Liu, 2016). The concentrations of the volatile constituents were measured by the calibration curves of the GC-peak area and the amount ratios for the target analyse relative to 2-methyl-3-heptanone. The final results were expressed as µg volatile compounds/kg of the boiled pork. Each value represented the average of triplicate determinations. 2-methyl-3-heptanone was used as the internal standard without considering the calibration factors, that is, all calibration factors were considered to be 1.00. The involved equation can be written as follows:

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

Where C*i* is the concentration of the compound in the boiled pork and OTi is the odour threshold in water. OTi was obtained from the online database (<http://www.odour.org.uk>) and some references related to flavour. Compounds with OAV ≥ 1 were considered to be the main contributors to total flavour.

## *2.6.* *E-nose analysis*

In this study, the odour profile of different boiled pork samples was discriminated using a portable electronic nose (PEN3) that operates with an enrichment and desorption unit (EDU) from Win Muster Airsense Analytics, Inc. (Airsense, Germany). This instrument consisted of a sampling apparatus, a detector unit that contains ten metal oxide sensors (Gao, Liu, An, Zhang, Ma, & Cui, 2017),and pattern identification software for data recording and elaboration (Wang, Wang, Liu, & Liu, 2012). Table 3 - 1 lists all sensors and their major applicants.

**Table 3 - 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 sulphur organic compounds and terpenes. | Hydrogen sulphide, 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 sulphur organic compounds | Hydrogen sulphide, 1 ppm |
| 10 | W3S | Reacts on high concentrations, very sensitive to several compounds | Methane, 100 ppm |

Approximately 1.00 g of a boiled pork sample was added to a 10 ml glass vial. A filtered and dried air flow (99%, 300 ml/min) was used as a carrier gas for E-nose detection. The data acquisition period lasted for 60 s, and an additional 180 s was required for system rebalance. For each sample, the E-nose analysis was repeated three times for the same conditions.

## *2.7.* *Statistical analysis*

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

# 3. Results and discussion

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

### 3.1.1. Volatile composition of boiled pork

A total of 61 volatile components were identified in boiled pork from the fore leg and hind leg muscles from different pig breeds by SPME-GC-MS/O, as is shown in Table 3 - 2. These compounds can be classified into nine chemical families, including aldehydes (50.5%-65.7%, 25/61), alcohols (4.8%-10.3%, 7/61), ketones (0.4%-0.9%, 1/61), esters (2.8%-12.3%, 3/61), aromatics (0.6%-8.4%, 8/61), hydrocarbons (2.1%-5.7%, 8/61), furans (5.3%-7.7%, 3/61), N-containing compounds (3.9%-6.5%, 2/61) and S-containing compounds (4.0%-9.5%, 4/61). The results showed that most of these compounds have been reported in the three different pig breeds (Pan, Yang, Zhu, and Wu, 2014). Among them, the largest number of aldehydes were found in boiled pork, followed by hydrocarbons, and aromatic compounds. Moreover, aldehyde compounds, which accounted for greater than 50.0% of the total volatile compounds, were the most abundant in boiled pork.

The concentration ratios and quantities of each group of volatile compounds in boiled pork is presented in Table 3 - 2. For the pig breeds yielding the boiled pork, there were 52, 44 and 54 volatile compounds in DLY, SMX and TB, respectively. The proportions of aldehydes and ketones (64.2%-65.7% and 0.5%-0.9%, respectively) were highest in TB, and the ratios of ethers, furans and S-containing compounds (9.0%-12.3%, 6.7%-7.7% and 8.6%-9.5%, respectively) were the highest in SMX. In contrast, aromatic compounds (7.7%-8.4%) were the most abundant in DLY. Owing to the main flavour of pork from aldehydes, furans and S-containing compounds and their presence in TB and SMX, TB and SMX had significant contributions to overall flavour. This result is in accordance with the study of Zhao et al., (2017). With respect to the parts for boiled pork, the major volatile components in the fore leg and hind leg muscles of DLY were aldehydes, alcohol and aromatics, which accounted for total concentrations of 55.1%-56.5%, 8.6%-10.3% and 6.6%-8.8%, respectively. The abundant volatiles in fore leg and hind leg muscles from SMX were aldehydes, ethers and S-containing compounds, which maintained the relationship of aldehydes > ethers > S-containing compounds. The main volatile compounds in fore leg and hind leg muscles from TB were aldehydes (64.2%-65.7%) and S-containing compounds (6.2%-8.1%). These analyses concluded that aldehydes and S-containing compounds had a dominant role in cooked pork (Aaslyng & Meinert 2017).

**Table 3 - 2:** Concentration ratios and quantities of volatile composition of boiled pork from different breeds of pigs.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Classes of components | Ratio% (quantities) | | | | | | | | | | | | |
| Fore leg muscle | | |  | Hind leg muscle | | |  | Fore leg and hind leg muscle | | |  | Breeds and parts |
| DLY | SMX | TB |  | 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.

Qualitative and quantitative analyses of the volatile components in boiled pork from different pig breeds are listed in Table 3 - 3. Aldehyde compounds, similar to the important volatile compounds in all types of meat products, were produced primarily by lipid oxidation and degradation reactions. Strecker degradation products of amino acids (Zhao et al., 2017; Li, Li, Zhang, Wang, Tang, and Chen, 2016) are also known to be major contributors to the unique flavour of cooked pork due to their low odour threshold (Lorenzo & Fonseca 2014). In this study, aldehydes were the most abundant groups and had the highest number of compounds in boiled pork samples. Eight of these compounds were simultaneously detected in all the boiled pork samples, including four alkenals (hexanal, heptanal, nonanal and hexadecanal), two alkadienals ((*E*)-2-octenal and (*E*)-2-nonenal) and two phenyl-containing aldehydes (benzaldehyde and 4-ethylbenzaldehyde). The four alkenals and two alkadienals are unsaturated fatty acid degradation products (Karahadian & Lindsay 1989). Meanwhile, the two phenyl-containing aldehydes are usually derived from the Strecker reaction (MacLeod, Ames, & Betz, 1988). Hexanal was the most abundant aldehyde and presented grassy notes, while (*E*)-2-octenal had a low odour threshold (3 μg·kg-1) and was described as having fatty notes (Gu, Wang, Tao, & Wu, 2013; Wang et al., 2018). Moreover, the hexanal and (*E*)-2-octenal contents in DLY were significantly higher (*P < 0.01*) than in SMX and TB, indicating that the extent of lipid oxidation in DLY was greater. The nonanal and benzaldehyde contents in TB were significantly higher (*P < 0.001*) than in DLY and SMX. This showed that TB had an advantage in the contribution to fruity and floral notes. Additionally, (*E*, *E*)-2,4-heptadienal and 9,12,15-octadecatrienal were exclusively found in boiled pork from SMX and TB and promoted a sweeter and fruit aroma (Allen & Hamilton 1989).

Alcohols are mainly generated by the oxidative decomposition of lipids (Zou, Kang, Liu, Qi, Zhou, & Zhang, 2018). Compared with short straight chain alcohols, long chain alcohols are considered to have more contributions to the aroma of meat products due to their lower odour thresholds (Li, Li, Zhang, Wang, Tang, & Chen, 2016). Seven alcohols were detected in this study, including three straight chain alcohols (1-pentanol, 1-hexanol and 1-octanol) and four branched chain alcohols (1-octen-3-ol, 2-hexyldecanol, (*E*)-2-octen-1-ol and anethole). Among these volatile compounds, 1-octen-3-ol, 1-octanol and (*E*)-2-octen-1-ol were found in all three varieties of boiled pork. The average contents of 1-octen-3-ol, with mushroom notes, and (*E*)-2-octen-1-ol, with green apple notes, in boiled pork from DLY were significantly (*P < 0.01*) higher than those from TB and SMX. Moreover, 2-hexyldecanol was only present in DLY, which indicated that it contributes more pleasant fruity and floral aromas (Petričević, Radovčić, Lukić, Listeš, & Medić, 2018) to overall flavour.

Furan, nitrogen and sulphur-containing compounds are well known as important heterocyclic compounds in meat products (Wang et al., 2018). Among the three furan compounds, 2-pentylfuran, with a fruity and buttery odour, had the highest contents (108.5-244.0 μg·kg-1) in all the boiled pork, which could be due to linoleic acid oxidization (Aparicio, Morales, & Alonso, 1996). 2-ethylfuran and 2-furanmethanol usually have pungent and caramel odours and have been reported in cooked meat (Gu, Wang, Tao, & Wu, 2013; Yang, Pan, Zhu, & Zou, 2014). For two nitrogen-containing compounds, the contents of pyridine and 2-acetylpyrazine were significantly higher (*P < 0.01*) in boiled meat from pig fore legmuscle than in the boiled meat from pig hind leg muscle. Furthermore, 3-methylthiophene and benzothiazole were very abundant in the boiled meat from pig hind leg muscle, while the amounts of dimethyl disulphide and 2-acetylthiazole identified in TB were greater than in DLY and SMX. The comparative analysis indicated that these four sulphur-containing compounds in boiled pork of hind leg muscle in TB are regarded as the major contributor to the cooked cabbage and roasted flavours (Zhou, Chong, Ding, Gu, & Liu, 2016). Previous reported noted that these compounds might originate from sulphur amino acids (free, peptides and proteinic amino acids), thiamine or glutathione (Girard & Durance, 2010).

Eight aromatic hydrocarbons and eight aliphatic hydrocarbons were identified in all the boiled pork samples. All of these compounds are usually formed by lipid oxidation (Kang, Gao, Ge, Zhou, & Zhang, 2017). Hydrocarbons had few effects on the aromatic profiles of meat products due to their high odour thresholds (Qi, Liu, Zhou, & Xu, 2017). Compared to boiled pork from SMX and TB, there were more and greater amounts of hydrocarbons in boiled pork from DLY, which may be due to the higher levels of lipid oxidation. Ester compounds can be formed by the esterification of acids and alcohols. A previous study showed that short-chain esters have fruity notes and long-chain esters have fatty notes (Wang et al., 2018). Terpinyl acetate and ethyl hexanoate were only found in SMX and may be used to distinguish boiled pork from different pig breeds. Vinyl hexanoate was present in all the investigated boiled pork samples.

**Table 3 - 3:** Identification and quantification of volatile compounds in boiled pork from different breeds of pigs by GC-MS/O (μg·kg-1).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compounds | 1DB-Wax | 2DB-5 | 3Identification | Fore leg muscle | | | | |  | Hind leg muscle | | | | |  | Sign. parts | | |
| DLY | SMX | TB | SEM | Sign. |  | DLY | SMX | TB | SEM | Sign. |  | DLY | SMX | TB |
| **Aldehydes (25)** |  |  |  | 3506.0ax | 1626.5cy | 2832.6by | 275.4 | \*\*\* |  | 2158.4by | 2023.7bx | 3511.9ax | 238.7 | \*\*\* |  | \*\*\* | \*\* | \*\*\* |
| 2,3-Dimethylpentanal | <900 | - | MS,RI | 0.0by | 0.0by | 13.8ax | 2.3 | \*\*\* |  | 2.8cx | 4.7bx | 13.0ax | 1.6 | \*\*\* |  | \*\*\* | \*\*\* | NS |
| Pentanal | 927 | 702 | MS,RI,O | 41.7by | 11.7cy | 145.3ax | 20.2 | \*\*\* |  | 60.9ax | 26.0bx | 0.0cy | 8.8 | \*\*\* |  | \*\* | \*\*\* | \*\*\* |
| Hexanal | 1074 | 797 | MS,RI,O | 2620.9ax | 1128.3cy | 1509.5by | 224.5 | \*\*\* |  | 1373.3ay | 1341.1ax | 1069.6bx | 51.1 | \*\* |  | \*\*\* | \* | \*\* |
| Heptanal | 1175 | 900 | MS,RI,O | 125.2ax | 56.9cx | 96.7bx | 10.2 | \*\*\* |  | 85.3by | 62.0cx | 91.6ax | 4.6 | \*\*\* |  | \*\* | NS | NS |
| Octanal | 1281 | 1002 | MS,RI,O | 82.2bx | 0.0cy | 87.0ax | 14.1 | \*\*\* |  | 57.0cy | 62.2bx | 85.1ay | 4.4 | \*\*\* |  | \*\*\* | \*\*\* | \* |
| Nonanal | 1388 | 1112 | MS,RI,O | 333.8bx | 255.7cx | 454.6ax | 29.4 | \*\*\* |  | 267.4by | 248.7bx | 362.6ay | 18.1 | \*\*\* |  | \*\* | NS | \*\* |
| (*E*)-2-Octenal | 1424 | 1062 | MS,RI,O | 47.2ax | 21.4bx | 23.0bx | 4.2 | \*\*\* |  | 31.3ay | 23.3cx | 27.0bx | 1.3 | \*\* |  | \*\* | NS | NS |
| (*E,E*)-2,4-Heptadienal | 1486 | 1015 | MS,RI,O | 0.0b | 0.0b | 9.2ay | 1.5 | \*\*\* |  | 0.0b | 0.0by | 10.8ax | 1.8 | \*\*\* |  | NS | NS | \*\* |
| Decanal | 1494 | 1210 | MS,RI,O | 0.0by | 11.0ax | 0.0by | 1.8 | \*\*\* |  | 21.7ax | 0.0cy | 13.8bx | 3.2 | \*\*\* |  | \*\*\* | \*\*\* | \*\*\* |
| Benzaldehyde | 1514 | 926 | MS,RI,O | 135.9bx | 60.2cy | 294.5ax | 34.5 | \*\*\* |  | 107.2by | 113.0bx | 276.3ay | 27.8 | \*\*\* |  | \*\* | \*\*\* | \* |
| (*E*)-2-Nonenal | 1531 | 1165 | MS,RI,O | 16.6ax | 8.7cy | 13.9by | 1.2 | \*\*\* |  | 14.2by | 13.0bx | 18.6ax | 0.9 | \*\*\* |  | \*\*\* | \*\* | \*\*\* |
| cis-4-Decenal | 1534 | 1202 | MS,RI | 22.4ax | 19.2bx | 0.0cy | 3.5 | \*\*\* |  | 24.0ax | 13.5by | 15.2bx | 1.7 | \*\*\* |  | NS | \*\*\* | \*\*\* |
| (*E*)-2-Decenal | 1600 | 1265 | MS,RI,O | 0.0b | 13.6ay | 0.0by | 2.3 | \*\*\* |  | 0.0c | 18.1ax | 14.2bx | 2.8 | \*\*\* |  | NS | \*\* | \*\*\* |
| 2-Butyl-2-octenal | 1665 | 1392 | MS,RI | 13.3a | 7.0bx | 0.0c | 1.9 | \*\*\* |  | 20.7b | 7.2cx | 21.3a | 2.3 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| 9,12,15-Octadecatrienal | 1675 | - | MS,RI | 0.0b | 5.3ax | 0.0b | 0.9 | \*\*\* |  | 0.0 | 0.0y | 0.0 | 0.0 | NS |  | NS | \*\*\* | NS |
| (*E,E*)-2,4-Nonadienal | 1695 | 1219 | MS,RI,O | 8.9ax | 4.1bx | 0.0cy | 1.3 | \*\*\* |  | 7.6ax | 4.1bx | 7.4ax | 0.6 | \*\* |  | NS | NS | \*\* |
| 4-Ethylbenzaldehyde | 1701 | 1173 | MS,RI | 10.8bx | 5.5cy | 33.5ay | 4.3 | \*\*\* |  | 9.1bx | 9.4bx | 69.5ax | 10.0 | \*\*\* |  | NS | \* | \*\*\* |
| Dodecanal | 1706 | 1418 | MS,RI,O | 4.5ay | 2.9by | 0.0cy | 0.7 | \*\*\* |  | 7.2bx | 6.0bx | 23.6ax | 2.8 | \*\*\* |  | \*\* | \*\* | \*\*\* |
| 2-Undecenal | 1747 | 1153 | MS,RI,O | 7.3ax | 0.0b | 0.0by | 1.2 | \*\*\* |  | 0.0by | 0.0b | 19.9ax | 3.5 | \*\* |  | \*\*\* | NS | \*\* |
| (*E,E*)-2,4-Decadienal | 1758 | 1324 | MS,RI,O | 11.4ax | 5.8bx | 0.0cy | 1.7 | \*\*\* |  | 4.0by | 0.0cy | 7.5ax | 1.1 | \*\*\* |  | \*\* | \*\* | \*\*\* |
| Tridecanal | 1812 | 1525 | MS,RI | 0.0by | 3.9ay | 0.0by | 0.7 | \*\*\* |  | 10.8bx | 12.1bx | 67.0ax | 9.3 | \*\*\* |  | \*\*\* | \*\*\* | \*\*\* |
| Tetradecanal | 1918 | 1626 | MS,RI | 0.0y | 0.0 | 0.0y | 0.0 | NS |  | 9.4bx | 0.0c | 85.1ax | 13.5 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| 4-Pentylbenzaldehyde | 1999 | 1472 | MS,RI | 0.0y | 0.0 | 0.0y | 0.0 | NS |  | 2.0bx | 0.0c | 11.3ax | 1.8 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| Pentadecanal | 2025 | 1712 | MS,RI | 0.0 | 0.0 | 0.0y | 0.0 | NS |  | 0.0b | 0.0b | 211.8ax | 35.3 | \*\*\* |  | NS | NS | \*\*\* |
| Hexadecanal | 2132 | 1832 | MS,RI | 24.0by | 5.3by | 151.5ay | 23.2 | \*\*\* |  | 42.4cx | 59.2bx | 989.6ax | 156.5 | \*\*\* |  | \*\* | \*\*\* | \*\*\* |
| **Alcohols (7)** |  |  |  | 534.2ax | 298.6bx | 208.9cy | 48.7 | \*\*\* |  | 405.1ay | 305.5bx | 331.8bx | 15.9 | \*\* |  | \*\* | NS | \*\* |
| 1-Pentanol | 1245 | 756 | MS,RI,O | 72.7ax | 32.0bx | 31.4bx | 6.9 | \*\*\* |  | 37.3ay | 0.0by | 0.0by | 6.2 | \*\*\* |  | \*\*\* | \*\*\* | \*\*\* |
| 1-Hexanol | 1347 | 865 | MS,RI,O | 20.7ax | 0.0c | 16.2by | 3.1 | \*\*\* |  | 0.0by | 0.0b | 116.1ax | 19.4 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| 1-Octen-3-ol | 1443 | 981 | MS,RI,O | 388.6ax | 228.9by | 113.7cy | 40.0 | \*\*\* |  | 306.4ay | 257.8bx | 155.0cx | 22.9 | \*\*\* |  | \*\* | \* | \* |
| 2-Hexyldecanol | 1546 | - | MS,RI | 0.0y | 0.0 | 0.0 | 0.0 | NS |  | 5.3ax | 0.0b | 0.0b | 0.9 | \*\*\* |  | \*\*\* | NS | NS |
| 1-Octanol | 1552 | 1072 | MS,RI,O | 19.6ax | 13.3by | 19.3ax | 1.1 | \*\* |  | 20.3bx | 22.5ax | 19.7bx | 0.5 | \* |  | NS | \*\*\* | NS |
| (*E*)-2-Octen-1-ol | 1608 | 1079 | MS,RI,O | 32.7ax | 24.4bx | 13.6cy | 2.8 | \*\*\* |  | 30.6ax | 25.2bx | 19.0cx | 1.8 | \*\* |  | NS | NS | \*\* |
| Anethole | 1818 | - | MS,RI,O | 0.0by | 0.0b | 14.7ay | 2.5 | \*\*\* |  | 5.2bx | 0.0c | 22.0ax | 3.3 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| **Ketones (1)** |  |  |  | 35.0ax | 13.6cx | 23.6by | 3.1 | \*\*\* |  | 16.8by | 16.3bx | 47.3ax | 5.1 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| (*E,Z*)-3,5-Octadien-2-one | 1564 | 1094 | MS,RI,O | 35.0ax | 13.6cx | 23.6by | 3.1 | \*\*\* |  | 16.8by | 16.3bx | 47.3ax | 5.1 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| **Esters (3)** |  |  |  | 498.7ax | 398.8bx | 156.2cx | 51.3 | \*\*\* |  | 259.2by | 322.7ay | 155.1cx | 25.0 | \*\*\* |  | \*\*\* | \*\* | NS |
| Terpinyl acetate | 1186 | 1361 | MS,RI,O | 0.0b | 18.1ax | 0.0b | 3.0 | \*\*\* |  | 0.0 | 0.0y | 0.0 | 0.0 | NS |  | NS | \*\*\* | NS |
| Ethyl hexanoate | 1225 | 1006 | MS,RI,O | 0.0b | 7.8ay | 0.0b | 1.3 | \*\*\* |  | 0.0b | 8.9ax | 0.0b | 1.5 | \*\*\* |  | NS | \*\* | NS |
| Vinyl hexanoate | 1313 | - | MS,RI | 498.7ax | 372.9bx | 156.2cx | 50.3 | \*\*\* |  | 259.2by | 313.8ay | 155.1cx | 23.8 | \*\*\* |  | \*\*\* | \*\* | NS |
| **Aromatics (8)** |  |  |  | 523.0ax | 19.2cy | 148.0by | 75.6 | \*\*\* |  | 285.5ay | 34.0cx | 239.5bx | 38.7 | \*\*\* |  | \*\*\* | \*\*\* | \*\*\* |
| Ethylbenzene | 1112 | 872 | MS,RI,O | 47.8ax | 0.0b | 0.0by | 8.0 | \*\*\* |  | 24.9ay | 0.0c | 8.4bx | 3.7 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| p-Xylene | 1119 | 868 | MS,RI | 71.8ax | 0.0c | 34.7bx | 10.4 | \*\*\* |  | 40.5ay | 0.0c | 35.8bx | 6.4 | \*\*\* |  | \*\*\* | NS | NS |
| o-Xylene | 1126 | 888 | MS,RI | 96.9ax | 0.0b | 0.0by | 16.2 | \*\*\* |  | 56.5ay | 0.0c | 34.1bx | 8.2 | \*\*\* |  | \*\*\* | NS |  |
| Styrene | 1246 | 895 | MS,RI,O | 244.1ax | 0.0c | 91.3by | 35.6 | \*\*\* |  | 131.3ay | 0.0c | 111.7bx | 20.5 | \*\*\* |  | \*\*\* | NS | \*\* |
| 1,2,4-Trimethylbenzene | 1271 | 945 | MS,RI | 15.9ax | 0.0by | 0.0by | 2.7 | \*\*\* |  | 4.5by | 8.0ax | 4.5bx | 0.6 | \*\* |  | \*\*\* | \*\*\* | \*\* |
| 1,2,4,5-Tetramethylbenzene | 1418 | 1218 | MS,RI | 37.2ax | 15.0by | 0.0cy | 5.4 | \*\*\* |  | 21.9ay | 20.1ax | 13.2bx | 1.4 | \*\* |  | \*\* | \*\* | \*\*\* |
| Naphthalene | 1733 | - | MS,RI,O | 9.2bx | 4.2cy | 13.2ay | 1.3 | \*\*\* |  | 5.9by | 5.9bx | 18.6ax | 2.1 | \*\*\* |  | \*\* | \* | \*\* |
| 2-Methylnaphthalene | 1845 | - | MS,RI | 0.0b | 0.0b | 8.7ay | 1.5 | \*\*\* |  | 0.0b | 0.0b | 13.4ax | 2.2 | \*\*\* |  | NS | NS | \*\*\* |
| **Hydrocarbons (8)** |  |  |  | 212.2ax | 76.8cy | 91.5by | 21.5 | \*\*\* |  | 122.6by | 135.6bx | 314.2ax | 31.1 | \*\*\* |  | \*\*\* | \*\*\* | \*\*\* |
| Limonene | 1190 | 1032 | MS,RI,O | 0.0y | 0.0 | 0.0 | 0.0 | NS |  | 14.4ax | 0.0b | 0.0b | 2.4 | \*\*\* |  | \*\*\* | NS | NS |
| Dodecane | 1195 | 1104 | MS,RI,O | 39.7ax | 15.7bx | 0.0c | 5.8 | \*\*\* |  | 10.4ay | 0.0by | 0.0b | 1.7 | \*\*\* |  | \*\*\* | \*\*\* | NS |
| Tridecane | 1296 | 1301 | MS,RI | 42.7ax | 20.9cy | 24.6by | 3.4 | \*\*\* |  | 25.3by | 52.2ax | 43.1ax | 4.2 | \*\* |  | \*\* | \*\* | \*\* |
| 3-Methyltridecane | 1368 | - | MS,RI | 0.0y | 0.0 | 0.0 | 0.0 | NS |  | 6.4ax | 0.0b | 0.0b | 1.1 | \*\*\* |  | \*\*\* | NS | NS |
| Tetradecane | 1400 | 1401 | MS,RI | 103.3ax | 29.0cy | 32.5by | 12.1 | \*\*\* |  | 44.2by | 46.8bx | 94.8ax | 8.2 | \*\*\* |  | \*\*\* | \*\* | \*\*\* |
| Pentadecane | 1501 | 1500 | MS,RI | 26.5bx | 11.2cy | 34.4ay | 3.4 | \*\*\* |  | 21.8cy | 36.6bx | 138.2ax | 18.4 | \*\*\* |  | \*\* | \*\*\* | \*\*\* |
| Longifolene | 1574 | 1403 | MS,RI | 0.0 | 0.0 | 0.0y | 0.0 | NS |  | 0.0b | 0.0b | 16.4ax | 2.7 | \*\*\* |  | NS | NS | \*\*\* |
| Hexadecane | 1600 | 1600 | MS,RI | 0.0 | 0.0 | 0.0y | 0.0 | NS |  | 0.0b | 0.0b | 21.6ax | 3.6 | \*\*\* |  | NS | NS | \*\*\* |
| **Furans (3)** |  |  |  | 335.7ax | 251.5bx | 226.6cy | 16.9 | \*\*\* |  | 192.0cy | 240.0by | 316.3ax | 18.2 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| 2-Ethylfuran | 952 | 708 | MS,RI,O | 39.8bx | 42.5by | 52.2ax | 2.0 | \*\*\* |  | 41.1cx | 50.6ax | 47.7bx | 1.4 | \*\*\* |  | NS | \*\* | NS |
| 2-Pentylfuran | 1218 | 988 | MS,RI,O | 244.0ax | 162.6bx | 132.6cy | 17.0 | \*\*\* |  | 108.5cy | 155.5bx | 231.4ax | 18.0 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| 2-Furanmethanol | 1625 | 851 | MS,RI,O | 51.9ax | 46.5bx | 41.8cx | 1.5 | \*\*\* |  | 42.4ay | 33.9cy | 37.3by | 1.3 | \*\* |  | \*\* | \*\* | \* |
| **N-containing compounds (2)** |  |  |  | 311.0ax | 259.4cx | 278.3bx | 7.6 | \*\*\* |  | 233.5ay | 209.7by | 214.6by | 3.7 | \*\*\* |  | \*\*\* | \*\*\* | \*\*\* |
| Pyridine | 1156 | 751 | MS,RI,O | 223.0ax | 175.2cx | 183.6bx | 7.4 | \*\*\* |  | 182.7ay | 157.5by | 152.8cy | 4.7 | \*\*\* |  | \*\*\* | \*\* | \*\*\* |
| 2-Acetylpyrazine | 1978 | 1095 | MS,RI,O | 88.0bx | 84.3cx | 94.7ax | 1.5 | \*\*\* |  | 50.8by | 52.2by | 61.8ay | 1.8 | \*\*\* |  | \*\*\* | \*\*\* | \*\*\* |
| **S-containing compounds (4)** |  |  |  | 246.4cx | 309.1b | 347.5a | 14.8 | \*\*\* |  | 243.7cx | 310.4b | 341.8a | 14.5 | \*\*\* |  | NS | NS | NS |
| Dimethyl disulphide | 1109 | 785 | MS,RI,O | 84.5cx | 128.2bx | 155.5ax | 10.4 | \*\*\* |  | 54.0cy | 116.5by | 140.2ay | 12.9 | \*\*\* |  | \*\* | \*\* | \*\*\* |
| 3-Methylthiophene | 1185 | 773 | MS,RI,O | 20.3ay | 17.2by | 18.2aby | 0.6 | \*\*\* |  | 25.7ax | 27.4ax | 25.4ax | 0.5 | NS |  | \*\* | \*\* | \*\* |
| 2-Acetylthiazole | 1632 | 1016 | MS,RI,O | 32.8by | 68.3ax | 69.5ax | 6.0 | \*\*\* |  | 42.6bx | 49.9ay | 51.3ay | 1.4 | \*\*\* |  | \*\* | \*\*\* | \*\*\* |
| Benzothiazole | 1934 | 1225 | MS,RI,O | 108.7ay | 95.4cy | 104.3by | 2.0 | \*\*\* |  | 121.5abx | 116.7bx | 124.9ax | 1.5 | \*  8  8 |  | \*\*\* | \*\*\* | \*\* |
| Total |  |  |  | 6202.1ax | 3253.6cy | 4313.2by | 431.5 | \*\*\* |  | 3916.6by | 3597.8cx | 5472.4ax | 291.0 | \*\*\* |  | \*\*\* | \* | \*\*\* |

***Note:*** DLY, Duroc × (Landrace × Yorkshire); SMX, Sanmenxia pig; TB, Tibetan pig. a-bmeans 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-ymeans in the same row not followed by a common superscript letter differ significantly (*P < 0.05,* Duncan test) (differences between fore leg and hind leg 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 indexes 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).

### 3.1.2. Odour-active compounds in boiled pork

The odour-active compounds in this study were defined as the compounds with OAVs > 1. The OAVs of odour-active compounds in boiled pork are presented in Table 3 - 4. Statistical analysis showed that the OAVs of 25 volatile compounds showed significant differences (*P < 0.05*) in boiled pork from the three pig breeds. Among the three varieties of boiled pork, TB contained the largest number of the odour-active constituents, including fourteen aldehydes, three alcohols, one hydrocarbon, two furans, one N-containing compound and three S-containing compounds (total of twenty-five), which indicated that TB displayed the most overall flavour among the meat samples. Compared with fore leg muscle from TB, there was a greater variety of odour-active compounds in hind leg muscle from TB. SMX contained 22 aroma-active constituents, which was the fewest odour-active compounds among the boiled pork from the three different pig breeds. For boiled pork from both the fore leg and hind leg muscles, the OAVs of half of the odour-active compounds did not show significant differences (*P > 0.05*), indicating that the muscles (fore leg and hind leg) presented similar flavour characteristics. In a word, the breed was considered as the main influencing factor for the overall flavour of boiled meat.

As shown in Table 3.4, the following seven odour-active constituents with relatively high OAVs were detected in all samples: hexanal (OAV at 213.9-524.2), nonanal (OAV at 248.7-454.6), 1-octen-3-ol (OAV at 56.9-194.3), dimethyl disulphide (OAV at 76.8-141.3), heptanal (OAV at 19.0-41.7), 2-pentylfuran (OAV at 18.1-40.7) and 2-ethylfuran (OAV at 17.3-22.9). These constituents were regarded as key odour-active compounds due to their significant contributions to the integral flavour. Linear aldehydes such as hexanal, nonanal and heptanal, with grass and fatty notes (Petričević, Radovčić, Lukić, Listeš, & Medić, 2018), come from lipid oxidation and may contribute to the overall flavour. 1-Octen-3-ol was the only alcohol among the odour-active compounds and has been reported to be generated by β-oxidation (Girard & Durance, 2010). Two furans, namely, 2-pentylfuran and 2-ethylfuran, might impart rubber and sweet flavours to the boiled pork, respectively. Dimethyl disulphide, with cooked cabbage notes, is an important fraction of aroma in fish paste products (Giri, Osako, & Ohshima, 2010).

**Table 3 - 4:** Odour-active compounds (OAVs > 1) in boiled pork from different breeds of pigs.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compounds | 1Odor thresholds  (μg·kg-1) | 2Odor descriptions | Fore leg muscle | | | | |  | Hind leg muscle | | | | |  | Sign. parts | | |
| DLY | SMX | TB | SEM | Sign. |  | DLY | SMX | TB | SEM | Sign. |  | DLY | SMX | TB |
| Pentanal | 9 | Fruity | 4.6by | 1.3cy | 16.1ax | 2.2 | \*\*\* |  | 6.8ax | 2.9bx | 0.0cy | 1.0 | \*\*\* |  | \*\* | \*\*\* | \*\*\* |
| Hexanal | 5 | Green, grass | 524.2ax | 225.7cy | 301.9bx | 44.9 | \*\*\* |  | 274.7ay | 268.2ax | 213.9by | 10.2 | \*\* |  | \*\*\* | \* | \*\* |
| Heptanal | 3 | Fatty, putty | 41.7ax | 19.0cx | 32.2bx | 3.4 | \*\*\* |  | 28.4by | 20.7cx | 30.5ax | 1.5 | \*\*\* |  | \*\* | NS | NS |
| Octanal | 0.578 | Fatty, pungent | 142.2bx | 0.0cy | 150.5ax | 24.4 | \*\*\* |  | 98.5cy | 107.6bx | 147.3ay | 7.5 | \*\*\* |  | \*\*\* | \*\*\* | \* |
| Nonanal | 1 | Fatty, floral, wax | 333.8bx | 255.7cx | 454.6ax | 29.4 | \*\*\* |  | 267.4by | 248.7bx | 362.6ay | 18.1 | \*\*\* |  | \*\* | NS | \*\* |
| (*E*)-2-Octenal | 3 | Burdock, fatty | 15.7ax | 7.1bx | 7.7bx | 1.4 | \*\*\* |  | 10.4ay | 7.8cx | 9.0bx | 0.4 | \*\* |  | \*\* | NS | NS |
| Decanal | 2 | Orange peel, soapy | 0.0by | 5.5ax | 0.0by | 0.9 | \*\*\* |  | 10.9ax | 0.0cy | 6.9bx | 1.6 | \*\*\* |  | \*\*\* | \*\*\* | \*\*\* |
| Benzaldehyde | 41.7 | Bitter, almond | 3.3bx | 1.4cy | 7.1ax | 0.8 | \*\*\* |  | 2.6by | 2.7bx | 6.6ay | 0.7 | \*\*\* |  | \*\* | \*\*\* | \* |
| (*E*)-2-Nonenal | 1 | Cardboard, cucumber | 16.6ax | 8.7cy | 13.9by | 1.2 | \*\*\* |  | 14.2by | 13.0bx | 18.6ax | 0.9 | \*\*\* |  | \*\*\* | \*\* | \*\*\* |
| (*E*)-2-Decenal | 0.4 | Fatty, green | 0.0b | 34.0ay | 0.0by | 5.7 | \*\*\* |  | 0.0c | 45.2ax | 35.5bx | 6.9 | \*\*\* |  | NS | \*\* | \*\*\* |
| (*E,E*)-2,4-Nonadienal | 0.16 | Fatty, green | 55.7ax | 25.7bx | 0.0cy | 8.2 | \*\*\* |  | 47.5ax | 25.3bx | 45.9ax | 4.0 | \*\* |  | NS | NS | \*\* |
| Dodecanal | 2 | Herbaceous, fatty | 2.2ay | 1.5by | 0.0cy | 0.3 | \*\*\* |  | 3.6bx | 3.0bx | 11.8ax | 1.4 | \*\*\* |  | \*\* | \*\* | \*\*\* |
| 2-Undecenal | 0.78 | Wax, fatty | 9.3ax | 0.0b | 0.0by | 1.6 | \*\*\* |  | 0.0by | 0.0b | 25.6ax | 4.5 | \*\* |  | \*\*\* | NS | \*\* |
| (*E,E*)-2,4-Decadienal | 0.07 | Fatty, deep-fried | 163.4ax | 83.1bx | 0.0cy | 24.0 | \*\*\* |  | 57.8by | 0.0cy | 107.0ax | 15.5 | \*\*\* |  | \*\* | \*\* | \*\*\* |
| 1-Octen-3-ol | 2 | Mushroom | 194.3ax | 114.5bx | 56.9cy | 20.0 | \*\*\* |  | 153.2ay | 128.9bx | 77.5cx | 11.5 | \*\*\* |  | \*\* | NS | \* |
| (*E*)-2-Octen-1-ol | 3 | Fruity, green apple | 10.9ax | 8.1bx | 4.5cy | 0.9 | \*\*\* |  | 10.2ax | 8.4bx | 6.3cx | 0.6 | \*\* |  | NS | NS | \*\* |
| Anethole | 15 | Rubber, paint | 0.0by | 0.0b | 1.0ay | 0.2 | \*\*\* |  | 0.3bx | 0.0c | 1.5ax | 0.2 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| Ethyl hexanoate | 1 | Fatty, green | 0.0b | 7.8ay | 0.0b | 1.3 | \*\*\* |  | 0.0b | 8.9ax | 0.0b | 1.5 | \*\*\* |  | NS | \*\* | NS |
| Styrene | 65 | Herbaceous, fatty | 3.8ax | 0.0c | 1.4by | 0.5 | \*\*\* |  | 2.0ay | 0.0c | 1.7bx | 0.3 | \*\*\* |  | \*\*\* | NS | \*\* |
| 2-Ethylfuran | 2.3 | Rubber, pungent | 17.3bx | 18.5by | 22.7ax | 0.8 | \*\*\* |  | 17.9cx | 22.0ax | 20.7bx | 0.6 | \*\*\* |  | NS | \*\* | NS |
| 2-Pentylfuran | 6 | Pungent, sweet | 40.7ax | 27.1bx | 22.1cy | 2.8 | \*\*\* |  | 18.1cy | 25.9bx | 38.6ax | 3.0 | \*\*\* |  | \*\*\* | NS | \*\*\* |
| Dimethyl disulphide | 1.1 | Cooked cabbage | 76.8cx | 116.5bx | 141.3ax | 9.4 | \*\*\* |  | 49.1cy | 105.9by | 127.4ay | 11.7 | \*\*\* |  | \*\* | \*\* | \*\*\* |
| 2-Acetylpyrazine | 62 | Nutty, popcorn | 1.4bx | 1.4bx | 1.5ax | 0.02 | \*\*\* |  | 0.8by | 0.8by | 1.0ay | 0.03 | \*\*\* |  | \*\*\* | \*\*\* | \*\*\* |
| 2-Acetylthiazole | 10 | Caramel, sweaty | 3.3by | 6.8ax | 7.0ax | 0.6 | \*\*\* |  | 4.3bx | 5.0ay | 5.1ay | 0.1 | \*\*\* |  | \*\* | \*\*\* | \*\*\* |
| Benzothiazole | 80 | Caramel, cheese | 1.4ay | 1.2cy | 1.3by | 0.03 | \*\*\* |  | 1.5bx | 1.5bx | 1.6ax | 0.02 | \* |  | \*\*\* | \*\*\* | \*\* |
| Total |  |  | 1662.6ax | 970.6cx | 1243.7bx | 100.9 | \*\*\* |  | 1080.0by | 1052.6bx | 1302.5ax | 40.8 | \*\*\* |  | \*\*\* | NS | NS |

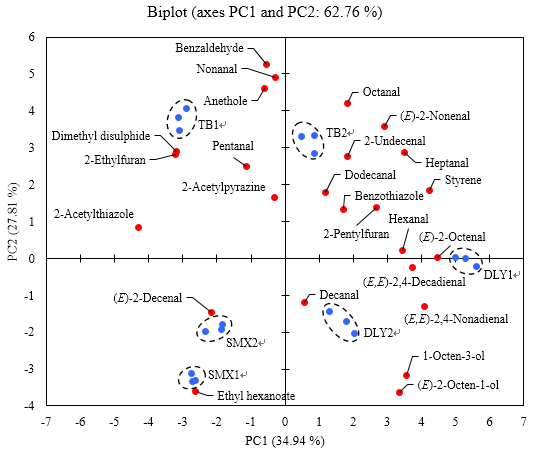
***Note:*** DLY, Duroc × (Landrace × Yorkshire); SMX, Sanmenxia pig; TB, Tibetan pig. a-bmeans in the same row not followed by a common subscript letter differ significantly (*P < 0.05,* Duncan test) (differences among the breeds of pigs). x-ymeans in the same row not followed by a common superscript letter differ significantly (*P < 0.05,* Duncan test) (differences between fore leg and hind leg 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).

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

To better visualize the data and reduce the dimensions of the original variables, PCA was performed to discriminate the boiled pork from the fore leg and hind leg muscles in the three pig breeds. Twenty-five odour-active compounds (OAVs > 1) were analysed by PCA. PCA scoring and a loading plot are presented in Figure 3 - 1. The first two principal components account for 34.94% and 27.81% of the variance, respectively, (62.76% in total). The six sample groups are clearly were well discriminated from one another. A clear separation between SMX and DLY can be observed for PC1, while TB was significantly different from SMX and DLY with respect to PC2. As shown in Figure 3 - 1 and Table 3 - 5, nine aldehydes (hexanal, r=0.727; heptanal, r=0.740; (*E*)-2-octenal, r=0.945; (*E*)-2-nonenal, r=0.617; (*E,E*)-2,4-nonadienal, r=0.846; (*E,E*)-2,4-decadienal, r=0.790; 1-octen-3-ol, r=0.747 and (*E*)-2-octen-1-ol, r=0.708) and one hydrocarbon (styrene, r=0.892) had high correlation coefficients with the positive side of PC1, which were present in DLY with high OAVs (Table 3.3). In contrast, only 2-acetylthiazole (r=-0.909), 2-ethlfuran (r=-0.677) and dimethyl disulphide (r=-0.671) showed high correlation coefficients with the negative side of PC1. Moreover, PC2 on the positive axis was highly influenced by benzaldehyde (r=0.988), nonanal (r=0.923), octanal (r=0.791), anethole (r=0.863) and (*E*)-2-nonenal (r=0.671), indicating that these compounds were the important odour-active compounds in TB (Figure 3 - 1), while PC2 on the negative axis was highly influenced by ethyl hexanoate and (*E*)-2-octen-1-ol (r=-0.682). Hence, (*E*)-2-nonenal was highly associated with the PC1 and PC2 positive axis and (*E*)-2-octen-1-ol was highly associated with the PC1 positive and PC2 negative axes. However, 2-acetylpyrazine and decanal were lowly relevant to the plane (correlation coefficients < 0.400). This suggested that these two compounds could not be described by PC1 and PC2. Additionally, according to the VIP scores in the PLS-DA analysis and p-values in the ANOVA of the studied odour-active compounds (Table 3 - 5), it could be concluded that a total of twelve volatile compounds with a VIP score > 1 and p-value < 0.05 were considered as potential flavour markers for the differentiation of boiled pork. These odour-active compounds were (*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.



**Figure 3 - 1:** PCA for odour-active compounds of the different boiled pork (TB1 = fore leg muscle of Tibetan pigs, TB2 = hind leg muscle of Tibetan pigs, DLY1 = fore legmuscle of Duroc × (Landrace × Yorkshire), DLY2 = hind leg muscle of Duroc × (Landrace × Yorkshire), SMX1 = fore legmuscle of Sanmenxia pigs, SMX2 = hind leg muscle of Sanmenxia pigs). The blue dots represent the samples from the different boiled pork, and the red dots respsent odour-active compounds.

**Table 3 - 5:** Information of PCA, PLS-DA and one-way analysis of variance.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Odour-active compounds | PCA | | PLS-DA | One-way analysis of variance |
| PC1 | PC2 | VIP score | *p* 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 |
| (*E*)-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 |
| (*E*)-2-Nonenal | 0.617 | 0.671 | 1.011 | < 0.01 |
| (*E*)-2-Decenal | -0.452 | -0.273 | 0.945 | < 0.01 |
| (*E, E*)-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 |
| (*E, E*)-2,4-Decadienal | 0.790 | -0.046 | 1.033 | < 0.01 |
| 1-Octen-3-ol | 0.747 | -0.598 | 1.039 | < 0.01 |
| (*E*)-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 |

AHC can be used to depict the similarities and differences among different boiled pork. Ward’s method with a metric of Euclidean distance was applied in this study. The results as a dendrogram are presented in Figure 3 - 2. The boiled meat samples were divided into three clusters. The third cluster included fore leg and hind leg muscles from SMX, with the lowest dissimilarity index, indicating that the fore leg and hind leg muscles from SMX have the most similar volatile profiles. Similarly, the second cluster, with fore leg and hind leg muscles from DLY, possessed similar volatile profiles. The first cluster consisted of fore leg and hind leg muscles from TB and had the highest dissimilarity index; this illustrated that the overall flavour of fore leg and hind leg muscles from TB was greatly different from that of the boiled pork from the other two pig varieties.

**Figure 3 - 2:** AHC results of the different boiled pork (TB1 = fore leg muscle of Tibetan pigs, TB2 = hind leg muscle of Tibetan pigs, DLY1 = fore legmuscle of Duroc × (Landrace × Yorkshire), DLY2 = hind leg muscle of Duroc × (Landrace × Yorkshire), SMX1 = fore legmuscle of Sanmenxia pigs, SMX2 = hind leg muscle of Sanmenxia pigs).

## *3.3. Volatile profiling of boiled pork using E-nose*

The signal from 10 sensors in response to volatile compounds from different boiled pork are presented in Figure 3 - 3. Figure 3 - 3a-c show that the responses of all ten sensors to boiled pork from fore leg and hind leg muscles had no significant differences, which explained their similar flavour. This also showed that W5S (broad-range nitrous oxides), W1W (terpenes and sulphur-containing organic compound), and W2W (aromatics and organic sulphides) had higher responses than other sensors, which suggested that they may contain more heterocyclic compounds, such as furans and N- and S-containing compounds. The response values of W1C and W3C were less than one. Both sensors were mainly sensitive to aroma components and ammonia. As shown in Figure 3 - 3d and e, the signals from the W5S and W1W sensors to the boiled pork from the three pig breeds obviously varied. Sensor W5S showed stronger responses to SMX, and while sensor W1W showed weaker responses to that breed. In a word, this result indicated that the influence of different pig breeds on flavour is greater than from different pig parts for boiled pork.

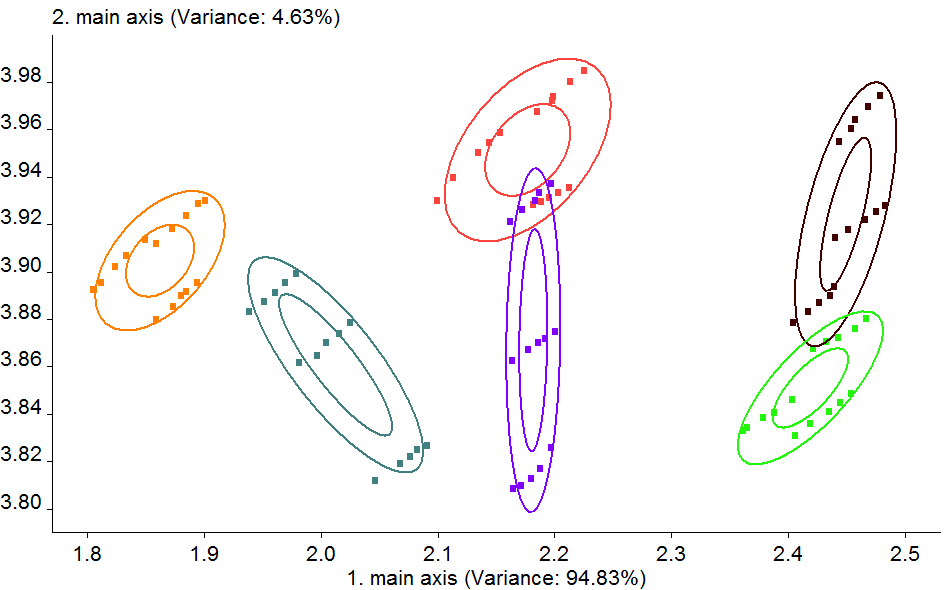
**b**

**Figure 3 - 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 = fore leg muscle of Tibetan pigs, TB2 = hind leg muscle of Tibetan pigs, DLY1 = fore legmuscle of Duroc × (Landrace × Yorkshire), DLY2 =hind leg muscle of Duroc × (Landrace × Yorkshire), SMX1 = fore legmuscle of Sanmenxia pigs, SMX2 = hind leg muscle of Sanmenxia pigs).

## *3.3.* *Discrimination of boiled pork by PCA and LDA*

E-nose analysis was performed to obtain a description of the odour profiles of boiled pork from different pig parts and breeds, and the PCA analysis results are shown in Figure 3 - 4a. The plot consists of two axes showing PC1 and PC2, which could explain 99.46% of the total variance. PC1 accounted for 94.83% and PC2 accounted for 4.63%. The contribution variance of PC1 and PC2 is over 90%, indicating that the first two PCs were sufficient to explain the maximum variation in the original types of boiled pork. According to Figure 3 - 4a, the dots corresponding to the fore leg and hind leg muscles from SMX and DLY had some overlap on PC1, and the sample points for the fore leg and hind leg muscles for TB were close to one another. Thus, the boiled pork samples can be divided into three groups (SMX, DLY and TB). This result illustrated that the boiled pork from different pig breeds had significantly different flavours, and that boiled pork from the fore leg and hind leg muscles of pigs had similar aroma compositions.

An LDA was also performed to investigate the similarities and differences among the six sample groups. As shown in Figure 3 - 4b, the first two PCs account for 88.58% of the variation in the data. Comparative analysis of all samples including each boiled pork sample could be clearly discriminated using LDA. The sample points for the fore leg and hind leg muscles from DLY and TB were close on PC1 (72.58%) and PC2 (16.00%), respectively. Moreover, the SMX fore leg and hind leg muscles clustered together on PC1 and PC2, which demonstrated that the odour profiles of the boiled pork from the fore leg and hind leg muscles of pigs appeared similar. The boiled pork from different pig breeds were far from one another. This demonstrated that there were considerable differences in their flavours. Both multivariate analyses (PCA and LDA) performed on E-nose data were suitable to distinguish the boiled pork based on its flavour profile.



**a**

SMX2

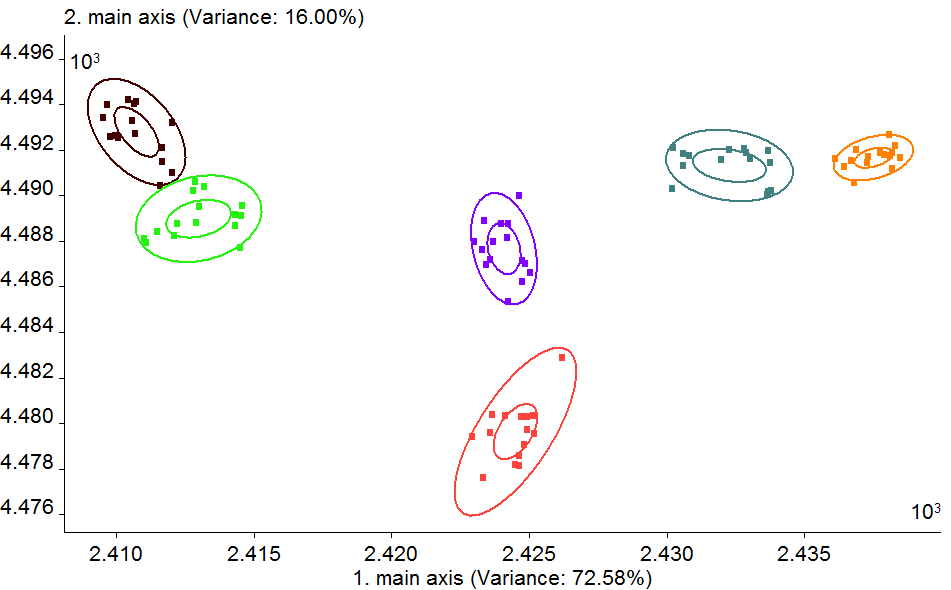
SMX1

DLY1

DLY2

TB1

TB2



**b**

SMX1

SMX2

DLY2

DLY1

TB1

TB2

**Figure 3 - 4:** PCA (a) and LDA (b) plot of e-nose response from different boiled pork (TB1 = fore leg muscle of Tibetan pigs, TB2 = hind leg muscle of Tibetan pigs, DLY1 = fore legmuscle of Duroc × (Landrace × Yorkshire), DLY2 = hind leg muscle of Duroc × (Landrace × Yorkshire), SMX1 = fore legmuscle of Sanmenxia pigs, SMX2 = hind leg muscle of Sanmenxia pigs).

# 4. Conclusions

In this study, a total of 61 volatile compounds were identified and quantified in boiled pork from different pig breeds by SPME-GC-MS/O. These compounds can be divided into nine categories: aldehydes, alcohols, ketones, esters, aromatics, hydrocarbons, furans, N- and S-containing compounds. The key odour-active volatiles from the evaluated samples were hexanal (OAV at 213.9-524.2), nonanal (OAV at 248.7-454.6), 1-octen-3-ol (OAV at 56.9-194.3), dimethyl disulphide (OAV at 76.8-141.3), heptanal (OAV at 19.0-41.7), 2-pentylfuran (OAV at 18.1-40.7) and 2-ethylfuran (OAV at 17.3-22.9), which significantly contribute to the overall flavour. Moreover, according to multicomponent statistics analyses, including PCA, PLS-DA, AHC and LDA, the boiled pork from different pig breeds could be classified into three separate groups. Twelve odour-active compounds were confirmed as potential flavour makers for the differentiation of boiled pork among the three pig breeds. Also, this study illustrated that the boiled pork from different pig breeds had significantly different flavours, and that boiled pork from the fore leg and hind leg muscles of pigs had similar aroma compositions.

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

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**4**

# Chapter Ⅳ. Effect of seasoning addition on volatile composition and sensory properties of stewed pork

*Spices are another important factor affecting the flavour of stewed pork. In order to give different flavour to the stewed pork, different spices are usually added during the stewing process of pork. This chapter investigated the volatile compounds of stewed pork with different seasoning recipes and also studied their influence on the flavour formation of stewed pork to better guide industrial production.*

This article will be submitted for publication in Food Chemistry.

**Abstract:** The objective of this study was to investigate the effect of different seasoning recipes (stewing pork with water, salt, spices, soy sauce, sugar and cooking wine, SP1-SP6) on volatile profiles and sensory evaluation of stewed pork. Volatile compounds were extracted by using solid phase microextraction and analysed by gas chromatography-olfactometry-mass spectrometry (GC-MS/O) and two-dimensional gas chromatographic combined with time-of-fight mass spectrometry (GC × GC-TOFMS). The stewed pork processed using SP1 and SP2 had the most abundant volatile compounds, especially aldehydes. This result indicated that the cooking pork with water and salt promoted lipid oxidation and amino acid degradation. As revealed by principal component analysis (PCA), samples SP3, SP4, SP5 and SP6 were close each other in PC1-PC2, whereas samples SP3 was located on the opposite side of samples SP4, SP5 and SP6 in PC1-PC3, which showed that the addition of spices had a significant influence on the flavour of stewed pork. Sensory evaluation revealed the stronger spicy, caramel and soy sauce odour were presented in samples SP3, SP4, SP5 and SP6. According to partial least squares regression (PLSR), hexanal, 1-octen-3-ol and 2-pentylfuran were highly associated with meaty and fatty odour, while some aldehydes and (*E*)-2-ocetn-1-ol were strongly and negatively correlated with spicy, caramel and soy sauce note.

**Keywords:** GC-MS/O; GC × GC-TOFMS; stewed pork; different seasoning recipes; PLSR

# 1. Introduction

According to the State Statistical Bureau of China report from 2014 to 2019, the pork production was about 54.0 million tons, accounting for more than 60% of the meat production and had the largest meat share. Stewed pork, a representative Chinese style meat product, is appreciated by consumers in most regions of China, due to its simple processing technique (Yang et al., 2019) and distinct flavour (Dong et al., 2019). It is often produced by stewing the fresh pork in water with various condiments and spices for a long time (Zeng et al., 2016). Due to the differences in the dietary habits of domestic consumers, the manufactures would add seasonings to make different flavoured stewed meat products to meet their needs well. It was also found that the seasonings create an enticing aroma during stewing and remove the undesirable odour in raw meat. Qin, Cai, Zhang, Liu, & Lai, (2019) reported that a total of 37 volatile compounds were identified in stewed meat broths and the main volatile compounds such as anethole, eucalptol, linalool, terpinen-4-ol, alpha-terpineol and cedrol may originated from star anise. The more hexadecanal, octadecanal and 9-octadecenal were present in soy sauce-stewed pork than those in water-boiled pork and only 3,5-dimethyl-*trans*-1,2,4-trithiolane was detected in soy sauce-stewed pork (Liu, Yang, & Wu, 2001). It is can be concluded that the addition of different food seasoning may have an important effect on the flavour formation of the meat products. Although it seems reasonable that the flavour of stewed meat products would be associated with the addition of different food seasonings, there is currently little quantitative information available with respect to the relationship between seasoning addition and the flavour of stewed meat.

To the best of our knowledge, the salt, spices, soy sauce, sugar and cooking wine are commonly used during cooking meat. Salt is a widely used additive in meat industry due to its preservation and antimicrobial properties (Overholt et al., 2016) and can accelerate lipid oxidation (Mariutti, & Bragagnolo, 2017) to generate some lipid-related volatile compounds, such as linear-chain aldehydes and furans (Feng, & Ahn, 2016) during the heating treatment of cooking. The soy sauce and cooking wine contain many amino acids which may be the critical contributors for the characteristic flavour of the stewed pork (Liu, Yang, & Wu, 2001; Wang, Hong, Ke, Hu, & Chen, 2017). The sugar, the flavour precursors to the Marillard reaction, usually plays an important role in the generation of aroma compounds of processed food. The studies had reported that the aldehydes, pyrazines and furans form Mailard reaction were considered as the key compounds of pot-stewed chicken meat products (Duan et al., 2015). The formation of methyl aldehyde and pyrazine may come from the degradation of amino acids, which were partly associated with the reaction between reducing sugars and amino acids (Olivares, Navarro, & Flores, 2011). The spices are used as flavouring ingredient and natural antioxidants (Lu, Kuhnle, & Cheng, 2018) in processed meat products. For example, the addition of 0.5% garlic or onion before irradiation was effective in reducing lipid oxidation and provided some garlic/onion aromas to the cooked ground beef (Yang, Lee, Moon, Paik, & Ahn, 2011). The addition of star anise changed the composition and proportion of volatile compounds, and imparted a spicy flavour to stewed chicken (Sun, Chen, Li, Liu, & Zhao, 2014). Although the generation and sources of volatile compounds from seasonings in meat products have been fully investigated, it is unknown that the changes and formation of flavour compounds produced by adding seasonings in the pork stewing process.

A combination of gas chromatography-mass spectrometry (GC-MS) and olfactometric detector is used to connect the volatile compounds with sensory sniffing to more favourably and effectively identify the key odour-active compounds from numerous volatile constituents in the meat products, such as stewed pork broth (Zhao et al., 2017) and braised pork (Song et al., 2019). Compared with gas chromatography-mass spectrometry/olfactometry (GC-MS/O), comprehensive two-dimensional gas chromatography-time-flight mass spectrometry (GC × GC-TOFMS) not only offers higher separation power, but also maintained more desirable sensitivity (Huang et al., 2019). The GC × GC-TOFMS has been consider a powerful technique for detailed profiling of the flavour profile of different food stuffs, such as braised chicken (Duan et al., 2015), dry-cured ham (Wang et al., 2018) and green teas (Zhu et al., 2018). Additionally, the key characteristic compounds and volatile profiles of the fresh and grilled eel were investigated by electronic nose (E-nose), GC-O, GC-MS and GC × GC-TOFMS (Huang et al., 2019). In summary, the combination of GC-MS/O and GC × GC-TOFMS is meaningful and facilitate the identification of major flavour compounds and analysis of flavour fingerprints.

The present study aimed to characterize the volatile profiles of stewed pork via GC-MS/O combined with GC × GC-TOFMS, and evaluate the effect of the addition of water, salt, spices, soy sauce, sugar and cooking wine on the volatile compounds and sensory properties of stewed pork. Moreover, this study also understood the changes of flavour compounds in stewed pork with the increase of seasonings, and explored the relationship between sensory evaluation and odour-active compounds in different stewed pork samples. Based on our finding, the different seasonings formula could be used as a valuable direction to produce the stewed pork with desirable flavour for consumers.

# 2. Materials and methods

## *2.1. Sampling and chemicals*

A total of 42 pieces of hind leg muscles with an average weight of approximately 0.8-1.0 kg from Duroc × (Landrace × Yorkshire) pig breed (aged 5-6 months and with body weights of 90-95 kg) were obtained from Chuying Agro-Pastoral Group Co. Ltd. (Zhengzhou, Henan Province, China). All pigs were reared under the same conditions, provided with the same feed and slaughtered following routine abattoir procedures (stunned, exsanguinated, scalded, dehaired and eviscerate). The hind leg muscles from the carcasses were cut into strips (6.0 cm × 4.0 cm × 10.0 cm) after removing visible fat and connective tissues. Then these muscles were packed in low-density polyethylene bags and stored at -20 °C. The sampling procedures were approved by the Animal Care and Use Committee of the Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, and performed in accordance with animal welfare and ethics. The 2-methyl-3-heptanone (99%) and *n*-alkanes (C7-C30) were of chromatographic grade and bought from Sigma-Aldrich (Shanghai, China)

## *2.2. Preparation of stewed pork samples*

### 2.2.1. Preparation of spice bag

The shallot (45 g/kg) and ginger (30 g/kg) were chopped evenly into pieces by knife. The spices were prepared by mixing 9 g/kg pepper, 1.2 g/kg Chinese cinnamon, 0.3 g/kg clove, 1.0 g/kg nutmeg, 0.5 g/kg licorice, 1.0 g/kg aniseed, 0.3g/kg cassia bark, 0.2g/kg cardamom, 1.0 g/kg fennel, 0.2 g/kg round cardamom, 1.2 g/kg tangerine peel, 0.8 g/kg galangal, 0.4 g/kg poncirus trifoliate, 0.6 g/kg costus root, 0.3 g/kg hawthorn, 0.4 g/kg long pepper, 0.5 g/kg rhizoma kaempferiae, 0.5 g/kg fructus tsaoko and 1.2 g/kg angelica dahurica (per kg of pork) and ground into powder by the high-speed grinder. One spice bag containing the above materials was prepared for stewed pork.

### 2.2.2. Seasoning formulations and stewing

The pork strips were boiled in water for 10 min at 100 °C to remove blood, and then stewed for 45 min at 98 ± 2 °C in different seasoning recipes, finally soaked for 60 min. The flow diagram of the stewed pork is shown in Figure 4 - 1. For stewing, the ratio of pork to stewing solution was 1:2 (w/v). The pork strips were divided into six stewing formulations to which the following treatments were randomly assigned (SP1: 2 L/kg water, SP2: 2 L/kg water + 60 g/kg salt, SP3: 2 L/kg water + 60 g/kg salt + one spice bag, SP4: 2 L/kg water + 60 g/kg salt + one spice bag + 20 g/kg soy sauce, SP5: 2 L/kg water + 60 g/kg salt + one spice bag + 20 g/kg soy sauce + 30 g/kg sugar, SP6: 2 L/kg water + 60 g/kg salt + one spice bag + 20 g/kg soy sauce + 30 g/kg sugar + 30 g/kg cooking wine). Fresh pork (FP) without stewing was used as the control group. In this study, SP3 was stewed in the aged brine that referred to the eighth brine prepared according to the following procedure (Li et al., 2016). The pork was stewed in water by adding salt and spice bag. The first brine (fresh brine) was obtained by removing the spice bag and stewed pork. Subsequently, supplementing water, salt and spice bag into fresh brine, the second brine was obtained by the same procedures. Aged brine was eventually produced according to this cyclic process until the eighth cycle. The processing method of aged brine of SP4, SP5 and SP6 was the same as that of SP3. The only difference was the composition of aged brine, which contained more the soy sauce, sugar and cooking wine in order than SP3. All pork samples were collected the vacuum bags and stored at -20 °C until used.

42 pieces of hind leg muscles

6 pieces of hind leg muscles

36 pieces of hind leg muscles

Stewed for 45 min with different recipes

Boiled for 10 min in water

**Recipe 1:** 2 L/kg water;

**Recipe 2:** 2 L/kg water + 60 g/kg salt;

**Recipe 3:** 2 L/kg water + 60 g/kg salt + one spice bag;

**Recipe 4:** 2 L/kg water + 60 g/kg salt + one spice bag + 20 g/kg soy sauce;

**Recipe 5:** 2 L/kg water + 60 g/kg salt + one spice bag + 20 g/kg soy sauce + 30 g/kg sugar;

**Recipe 6:** 2 L/kg water + 60 g/kg salt + one spice bag + 20 g/kg soy sauce + 30 g/kg sugar + 30 g/kg cooking wine.

Soaked for 60 min

**Figure 4 - 1:** Flow diagram of the stewed pork. The processing procedures consist of three steps including boiling for 10 min, stewing for 45 min and soaked for 60 min. The sampling points were chosen according to different stewing recipes.

## *2.3. Extraction of volatile compounds*

The extraction of volatiles from the stewed pork was carried out using the manual solid-phase micro-extraction (SPME) equipped with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, Inc., Bellefonte, PA, USA). Briefly, 5.0 g of the pork sample was weighed precisely and placed in a 40 mL headspace vial. Immediately after, 1 μL of 2-methyl-3-heptanone (0.816 μg/μL) was added and sealed tightly with screw caps fitted with a Teflon/silicon septum. The vial was incubated in a thermostatic water bath at 60 °C for 20 min. The selected fibre was used to extract the volatile compounds in head space for 40 min at 60 °C. Upon completion, the fibre was inserted into the injection port (250 °C) of the GC instrument to desorb the analyses for 5 min. All samples were extracted in triplicate.

## *2.4. GC-MS/O analysis*

The method was performed by the method of (Han, Zhang, Fauconnier, & Mi, 2019) with minor modifications. The volatile compounds of stewed pork were analysed and identified by a gas chromatography-mass spectrometry (GC-MS) instrument (7890A-7000B, Agilent Technologise, Inc., Santa Clara, CA, USA) equipped with an olfactory detection port (Sniffer 9000; Brechbuhler, Schlieren, Switzerland). Capillary column DB-wax (30 m × 0.32 mm i.d., 0.25 µm film thickness; J & W Scientific, Inc., Folsom, CA, USA) was used with helium (purity of ≥99.999%) as the carrier gas at 1.2 mL/min flow rate. The front inlet temperature was 250 °C with a solvent delay of 4 min. The temperature program was as follows: Oven temperature was maintained at 40 °C for 3 min, ramped to 200 °C at a rate of 5 °C/min, then ramped to 240 °C at a rate of 10 °C/min with a 3 min final hold. The infector mode was splitless. The transfer line temperature and ion source temperature were kept a 240 °C and 230 °C. Electro-impact mass spectra were generated at 70eV, with *m/z* scan range from 50 to 400 amu. A sniffing port (Sniffer 9000) coupled to a GC-MS instrument was used for odour-active compound characterization. The effluent from the capillary column was split 1:1 (*v/v*) between the mass spectrometry detector and the olfactory detector port. A panel that contains eight trained staff was utilized for the sniffing test on GC-O. The volatile compounds were initially identified by the National Institute of Standards and Technology (NIST) Mass Spectral Library (Version 2.0). Subsequently, the identified compounds were further confirmed based on a comparison of GC retention indices (*RI*) with authentic compounds. Also, the qualitative analysis was also performed by odour properties.

## *2.5. GC × GC-TOFMS analysis*

The GC × GC-TOFMS system consist of an Agilent 7890 gas chromatography (Agilent Technologies, Palo Alto, CA, USA) equipped with cold-jet modulator and time-of-flight mass spectrometer (TOFMS; LECO Pegasus 4D). The first column was DB-WAX (30 m × 0.25 mm i.d. × 0.25 μm film thickness) and the second column was DB-17HT (2 m × 0.1 mm i.d. × 0.1 μm film thickness). GC × GC conditions: the temperature of the injection port was 240 °C; helium (99.999%) flow, 1.0 mL/min; splitless injection; 6.0 s of modulation period; the column temperature program for the 1st D column: initial temperature was 40 °C and held for 1 min, increased to 220 °C at 3.0 °C per min, to 230 °C at 10 °C per min and held for 5 min; the column temperature program of the 2nd D column: initial temperature at 45 °C (held for 1 min), increased to 225 °C at 3.0 °C per min (held for 2.5 min), to 230 °C at 10 °C per min (held for 5 min). TOF-MS conditions: The ion source and transfer line to the mass spectrometer were maintained at 220 °C and 290 °C, respectively. The ionization potential of MS was 70 eV, the detector voltage was 1620 V, the scan range was 33 to 450 m/z, and the mass spectra data acquisition rate was 50 spectra/s. The identification of volatile compounds was based on the NIST 2014 library (Hewlett-Packard Co.), the mass spectral match factor ≥ 800 and similarity ≥ 1000.

## *2.6. Quantification of volatile compounds*

The volatile compounds of stewed pork were semi-quantitated by the method of calibration with an internal standard (IS). The concentrations of the volatile constituent were measured by the calibration curves of the GC-peak area and the amount ratios for the target analyst relative to 2-methyl-3-heptanone. The quantitative data of the identified compounds were obtained without considering the calibration factors, that is, the calibration factors were considered to be 1.00. The concentration of each compound was calculated as follows:

(1)

The odour activity value (OAV) of a compound was calculated as the ratio of its centration in the stewed pork to its odour threshold in water. The equation was show below:

(2)

Where C*i* is known as the concentration of the compound in the stewed pork and OTi is the odour threshold in water. Compounds with OAV ≥ 1 were considered to be the main contributors to the total flavour.

## *2.7. Sensory evaluation*

Sensory evaluation was carried out by 8 trained panellists (4 females and 4 males, aged 25–35 years). All assessors were recruited from Chinese Academy of Agriculture, Beijing and had at least one year of experience in the descriptive analysis of stewed meat products. In order to be more familiar with the flavour characteristics of stewed pork, the assessors carried out 12 weeks of training sessions (2 times per week and 2 h per session). The panellist descripted and defined the flavour attributes, reference standard and intensities (Table 4 - 1). Samples were coded with three-digit randomized numbers and presented to the assessors at room temperature. Panellists selected five flavour attributes to be evaluated, namely, fatty odour, meaty odour, caramel odour, soy sauce odour and spicy odour. Each attribute was scored on a 10 cm non-structured lines with anchor point at each end (0 = not perceivable, 10 = strongly perceivable) (Wang, Song, Zhang, Tang, & Yu, 2016). The mean value of sensory attributes was showed in the radar chart.

**Table 4 - 1:** Information of the definitions and reference standards of odour attributes.

|  |  |  |
| --- | --- | --- |
| Odour attributes | Definitions | References (intensity) |
| Fatty | The smell associated with lard oil | Lard oil at 25°C (6.0) |
| Meaty | The smell associated with cooked pork | 20.0 g of defatted pork in 60.0 mL of water was boiled for 1 h (8.0) |
| Caramel | The smell associated with burning white sugar | 5.0 g of burning white sugar in 50.0 mL water (6.0) |
| Soy sauce | The smell associated with soy sauce | 3.0 g of defatted pork in 50.0 mL of water (7.0) |
| Spicy | The smell associated with mixed spices | Freshly ground mixed spices (8.0) |

## *2.8. Statistical analysis*

The content of all volatile compounds and OAVs of the odour-active compounds in stewed pork were expressed as the mean ± standard deviation (SD). Significant differences were determined by one-way analysis of variance (ANOVA) and Duncan’s multiple range test at *P < 0.05* of SPSS software (v. 19.0, SPSS, Inc., Chicago, IL, USA). The odour-active compounds and sensory evaluation were performed PCA, PLS-DA and PLSR using the software XLSTAT (2016) from Addinsoft (Barcelona, Spain) and the heat map of the correlation analysis were conducted in R v3.2.2 (R Studio Team, 2012).

# 3. Results and discussion

## *3.1. Analysis of volatile components by GC-MS/O and GC × GC-TOFMS*

The kinds and content ratios of volatile components in all pork samples were shown in Table 4 - 2. For the fresh and stewed pork, compared with compounds detected by GC-MS/O, more volatile compounds (e.g. aldehydes, alcohols, ketones, heterocyclic and sulphur-containing compounds) were identified by GC × GC-TOFMS. Some long-chain aldehydes and hydrocarbons, such as β-cyclocitral, tetradecanal, hexadecanal, decane, dodecane, tridecane, teradecane, pentadecane and longifolene, were not detected using GC × GC-TOFMS. These results showed that the GC × GC-TOFMS combined with GC-MS/O could more comprehensively analyse the volatile profile in the fresh and stewed pork. For the results of GC-MS/O analysis of volatile compounds (Table 4 - 3), aldehydes were the compound class present at the highest concentration, accounting for 34.9-66.8%, followed by hydrocarbons and alcohols (12.3-48.4% and 9.1-26.3%). With respect to GC × GC-TOFMS analysis, the abundant volatile compounds were aldehydes, alcohols, ketones, hydrocarbons, heterocyclic and sulphur-containing compounds in stewed pork.

**Table 4 - 2:** Identification of volatile compounds of the fresh and stewed pork by GC-MS/O and GC×GC-TOFMS.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Compounds | GC-MS/O | | |  | GC × GC-TOFMS | | | |  | Different processing methods | | | | | | |  | I method3 |
| RT (min) | RI1 | RI\*2 |  | PeakⅠ (min) | Peak Ⅱ (s) | Similarity match/  Reverse match | Library probability |  | FP | SP1 | SP2 | SP3 | SP4 | SP5 | SP6 |  |
|  | **Aldehydes (30)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 1 | Acetaldehyde |  |  |  |  | 5.2 | 1.64 | 945/972 | 21124 |  | B4 | N.D.5 | N.D. | N.D. | N.D. | B | B |  | MS |
| 2 | Propanal |  |  |  |  | 5.2 | 1.90 | 928/928 | 6177 |  | N.D. | N.D. | B | N.D. | B | B | B |  | MS |
| 3 | Butanal |  |  |  |  | 6.4 | 2.46 | 911/913 | 9365 |  | B | B | B | B | B | B | B |  | MS |
| 4 | Pentanal | 6.543 | 960 | 975 |  | 8.6 | 3.06 | 909/911 | 8986 |  | B | A+B6 | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 5 | Hexanal | 9.105 | 1065 | 1064 |  | 9.5 | 1.64 | 910/910 | 9236 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 6 | Heptanal | 11.928 | 1168 | 1182 |  | 16.0 | 3.34 | 910/910 | 8332 |  | B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 7 | Octanal | 14.881 | 1275 | 1287 |  | 20.5 | 3.50 | 937/944 | 9166 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 8 | (Z)-2-Heptenal |  |  |  |  | 21.8 | 3.42 | 894/906 | 4130 |  | N.D. | B | B | N.D. | N.D. | N.D. | N.D. |  | MS,O |
| 9 | 2-Methylpentanal |  |  |  |  | 24.8 | 4.28 | 866/892 | 6234 |  | N.D. | B | N.D. | B | N.D. | N.D. | B |  | MS |
| 10 | Nonanal | 17.671 | 1379 | 1388 |  | 25.0 | 3.28 | 918/918 | 7699 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 11 | (E)-2-Octenal | 18.574 | 1414 | 1425 |  | 26.4 | 3.22 | 917/918 | 5920 |  | B | A+B | A+B | B | B | B | B |  | MS,RI,O |
| 12 | Decanal |  |  |  |  | 29.4 | 3.86 | 897/898 | 6270 |  | B | B | B | B | B | B | B |  | MS,O |
| 13 | Benzaldehyde | 20.761 | 1502 | 1513 |  | 30.0 | 2.40 | 942/942 | 8474 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 14 | (E)-2-Nonenal |  |  |  |  | 30.8 | 3.16 | 921/921 | 6659 |  | B | B | B | B | B | B | B |  | MS,O |
| 15 | cis-4-Decenal | 21.280 | 1521 | 1544 |  | 31.1 | 3.42 | 882/885 | 6721 |  | B | A+B | A+B | B | B | B | B |  | MS,RI,O |
| 16 | Benzeneacetaldehyde |  |  |  |  | 34.8 | 2.42 | 921/937 | 7472 |  | B | B | B | B | B | B | B |  | MS,O |
| 17 | (E)-2-Decenal |  |  |  |  | 35.1 | 3.14 | 878/882 | 4859 |  | B | B | B | B | B | B | B |  | MS,O |
| 18 | 2-Butyl-2-octenal |  |  |  |  | 36.2 | 3.78 | 856/887 | 7631 |  | N.D. | B | B | N.D. | N.D. | N.D. | N.D. |  | MS |
| 19 | (E,E)-2,4-Nonadienal |  |  |  |  | 37.2 | 2.80 | 849/878 | 4069 |  | B | B | B | N.D. | N.D. | N.D. | N.D. |  | MS,O |
| 20 | 2-Undecenal |  |  |  |  | 39.2 | 3.10 | 864/869 | 2780 |  | B | B | B | N.D. | N.D. | N.D. | N.D. |  | MS |
| 21 | 4-(1-Methylethyl)-benzaldehyde | 26.715 | 1762 | 1759 |  | 40.0 | 2.78 | 869/872 | 7423 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 22 | (E,E)-2,4-Decadienal | 27.377 | 1791 | 1790 |  | 41.2 | 2.72 | 877/910 | 5000 |  | B | A+B | A+B | B | N.D. | B | B |  | MS,RI,O |
| 23 | p-Anisaldehyde | 31.554 | 1999 | 1982 |  | 48.0 | 2.34 | 898/900 | 7404 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 24 | Cinnamaldehyde | 31.834 | 2014 | 2040 |  | 48.5 | 2.32 | 856/903 | 3588 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 25 | Pentadecanal | 31.817 | 2009 | 2024 |  | 52.3 | 3.08 | 918/929 | 3455 |  | B | A+B | A+B | B | B | B | B |  | MS,RI |
| 26 | 10-Undecenal |  |  |  |  | 59.3 | 3.04 | 868/874 | 3564 |  | B | B | B | N.D. | N.D. | N.D. | N.D. |  | MS |
| 27 | 9-Octadecenal |  |  |  |  | 59.2 | 3.00 | 858/868 | 1959 |  | B | B | B | B | B | B | B |  | MS |
| 28 | β-Cyclocitral | 23.242 | 1606 | 1598 |  |  |  |  |  |  | A7 | N.D. | N.D. | N.D. | A | A | A |  | MS,RI,O |
| 29 | Tetradecanal | 29.739 | 1906 | 1927 |  |  |  |  |  |  | N.D. | A | A | A | N.D. | N.D. | N.D. |  | MS,RI,O |
| 30 | Hexadecanal | 33.813 | 2119 | 2137 |  |  |  |  |  |  | N.D. | A | A | A | A | A | A |  | MS,RI |
|  | **Alcohols (23)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 31 | 4-Methyl-1-pentanol |  |  |  |  | 4.2 | 1.78 | 830/992 | 6158 |  | N.D. | N.D. | B | N.D. | N.D. | B | B |  | MS |
| 32 | 1-Butanol |  |  |  |  | 14.8 | 2.04 | 869/906 | 6144 |  | B | N.D. | B | B | B | B | B |  | MS |
| 33 | 1-Penten-3-ol |  |  |  |  | 15.3 | 2.06 | 840/865 | 6976 |  | N.D. | B | B | N.D. | B | N.D. | N.D. |  | MS |
| 34 | 1,8-Cineole | 12.540 | 1197 | 1209 |  | 16.9 | 4.48 | 837/837 | 7603 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 35 | 1-Pentanol | 13.995 | 1242 | 1247 |  | 18.8 | 2.06 | 940/940 | 8241 |  | B | A+B | A+B | B | B | B | B |  | MS,RI,O |
| 36 | (Z)-2-Penten-1-ol |  |  |  |  | 21.4 | 2.12 | 847858 | 7080 |  | N.D. | B | B | N.D. | N.D. | N.D. | N.D. |  | MS |
| 37 | 1-Hexanol |  |  |  |  | 23.0 | 2.22 | 906/906 | 5249 |  | B | B | B | B | B | B | B |  | MS |
| 38 | 1-Octen-3-ol | 19.030 | 1432 | 1441 |  | 27.1 | 2.20 | 935/935 | 7624 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 39 | 1-Heptanol |  |  |  |  | 27.3 | 2.30 | 914/914 | 5292 |  | B | B | B | B | B | B | B |  | MS |
| 40 | 2-Ethyl-1-hexanol |  |  |  |  | 28.8 | 2.44 | 914/912 | 6606 |  | B | B | B | B | B | B | B |  | MS |
| 41 | Linalool | 21.400 | 1528 | 1545 |  | 31.2 | 2.42 | 908/908 | 7416 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 42 | 1-Octanol | 21.698 | 1541 | 1557 |  | 31.5 | 2.28 | 925/925 | 4288 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 43 | 2,3-Butanediol |  |  |  |  | 32.0 | 1.74 | 876/900 | 7628 |  | N.D. | N.D. | B | N.D. | B | N.D. | N.D. |  | MS |
| 44 | Terpinen-4-ol | 22.813 | 1588 | 1600 |  | 33.4 | 2.74 | 893/895 | 6364 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 45 | (E)-2-Octen-1-ol | 23.014 | 1596 | 1605 |  | 33.7 | 2.16 | 896/896 | 6115 |  | B | A+B | A+B | B | A+B | A+B | A+B |  | MS,RI,O |
| 46 | 1-Nonanol |  |  |  |  | 35.5 | 2.36 | 894/894 | 3409 |  | B | B | B | B | B | N.D. | B |  | MS |
| 47 | Terpineol | 24.924 | 1680 | 1688 |  | 36.9 | 2.54 | 926/929 | 7526 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 48 | trans-2-Undecen-1-ol |  |  |  |  | 41.7 | 3.46 | 857/880 | 1377 |  | B | B | B | B | B | N.D. | B |  | MS |
| 49 | (4R,6R)-cis-Carveol |  |  |  |  | 41.8 | 2.30 | 859/863 | 7329 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 50 | cis-Geraniol |  |  |  |  | 42.2 | 2.2 | 867/867 | 9728 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 51 | Phenylethyl alcohol | 29.313 | 1886 | 1903 |  | 44.2 | 2.00 | 901/907 | 7082 |  | B | N.D. | B | B | B | B | A+B |  | MS,RI,O |
| 52 | 1-Undecanol |  |  |  |  | 46.6 | 2.42 | 880/895 | 1095 |  | B | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |  | MS |
| 53 | 1-Dodecanol |  |  |  |  | 53.2 | 2.42 | 844/851 | 1226 |  | B | B | N.D. | N.D. | N.D. | N.D. | N.D. |  | MS |
|  | **Ketones (19)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 54 | 2-Butanone |  |  |  |  | 6.8 | 2.56 | 903/906 | 8450 |  | B | B | B | B | B | B | B |  | MS |
| 55 | 2,3-Butanedione |  |  |  |  | 8.5 | 2.06 | 954/977 | 7946 |  | B | B | B | B | N.D. | B | B |  | MS,O |
| 56 | 2,3-Pentanedione |  |  |  |  | 11.0 | 2.66 | 892/902 | 9222 |  | N.D. | B | B | N.D. | N.D. | B | B |  | MS |
| 57 | 2-Heptanone |  |  |  |  | 15.8 | 3.98 | 864/866 | 6987 |  | B | B | B | B | N.D. | B | B |  | MS |
| 58 | 3-Octanone |  |  |  |  | 19.0 | 4.70 | 864/871 | 6442 |  | B | B | B | N.D. | B | B | N.D. |  | MS |
| 59 | 2-Octanone |  |  |  |  | 20.3 | 1.82 | 825/836 | 3002 |  | B | B | B | N.D. | N.D. | N.D. | N.D. |  | MS |
| 60 | 1-Hydroxy-2-propanone |  |  |  |  | 20.9 | 1.86 | 925/925 | 7017 |  | N.D. | B | B | B | B | B | B |  | MS |
| 61 | 2,3-Octanedione |  |  |  |  | 21.9 | 3.22 | 876/878 | 7035 |  | B | B | B | B | B | N.D. | B |  | MS,O |
| 62 | 6-Methyl-5-hepten-2-one | 16.095 | 1319 | 1336 |  | 22.5 | 3.40 | 850/850 | 9200 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 63 | 3-Octen-2-one |  |  |  |  | 25.5 | 3.30 | 871/889 | 8621 |  | B | B | B | N.D. | N.D. | N.D. | N.D. |  | MS |
| 64 | (E,E)-3,5-Octadien-2-one |  |  |  |  | 32.1 | 2.80 | 864/976 | 8185 |  | N.D. | N.D. | B | N.D. | N.D. | N.D. | N.D. |  | MS |
| 65 | 6-Methyl-3,5-heptadiene-2-one |  |  |  |  | 32.9 | 2.92 | 826/849 | 4515 |  | N.D. | N.D. | B | N.D. | N.D. | B | N.D. |  | MS |
| 66 | Acetophenone |  |  |  |  | 35.0 | 2.58 | 886/898 | 4882 |  | B | B | B | B | B | B | B |  | MS |
| 67 | 4-Isopropyl-2-cyclohexenone |  |  |  |  | 35.9 | 3.08 | 861/873 | 5963 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 68 | Piperitone | 25.663 | 1713 | 1739 |  | 38.1 | 2.96 | 894/906 | 8141 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 69 | Carvone |  |  |  |  | 38.3 | 2.94 | 854/869 | 6159 |  | N.D. | N.D. | N.D. | B | B | N.D. | N.D. |  | MS |
| 70 | 4-Phenyl-2-butanone |  |  |  |  | 42.6 | 2.58 | 849/857 | 8665 |  | N.D. | N.D. | N.D. | B | N.D. | B | B |  | MS |
| 71 | 4-Methoxyphenylacetone |  |  |  |  | 52.2 | 2.34 | 803/825 | 7026 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 72 | Xanthoxylin | 40.619 | 2576 |  |  | 64.5 | 2.32 | 875/883 | 9405 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS |
|  | **Hydrocarbons (25)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 73 | 2-Methylbutane |  |  |  |  | 4.4 | 2.30 | 812/851 | 1512 |  | N.D. | B | N.D. | B | N.D. | B | B |  | MS |
| 74 | Benzene |  |  |  |  | 7.7 | 2.78 | 847/851 | 7126 |  | N.D. | N.D. | B | B | N.D. | N.D. | N.D. |  | MS |
| 75 | Toluene | 7.970 | 1022 | 1028 |  | 10.4 | 3.30 | 922/922 | 5019 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 76 | Ethylbenzene |  |  |  |  | 13.4 | 3.86 | 936/936 | 4866 |  | B | B | B | B | B | B | B |  | MS |
| 77 | 1,3-Dimethylbenzene |  |  |  |  | 13.4 | 5.76 | 914/921 | 4126 |  | B | B | B | B | B | B | B |  | MS |
| 78 | p-Xylene | 10.735 | 1125 | 1130 |  | 13.9 | 3.62 | 943/948 | 2826 |  | A | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 79 | o-Xylene | 11.953 | 1169 | 1183 |  | 14.0 | 3.56 | 947/947 | 3148 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 80 | D-Limonene | 12.411 | 1185 | 1192 |  | 16.3 | 5.48 | 899/900 | 7198 |  | N.D. | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 81 | Phellandrene |  |  |  |  | 16.8 | 5.98 | 864/867 | 4478 |  | N.D. | N.D. | N.D. | B | B | N.D. | N.D. |  | MS |
| 82 | (Z)-3,7-Dimethyl-1,3,6-octatriene |  |  |  |  | 18.7 | 4.78 | 873/874 | 3365 |  | N.D. | N.D. | N.D. | B | N.D. | B | N.D. |  | MS |
| 83 | 1,3,5,7-Cyclooctatetraene |  |  |  |  | 18.9 | 2.84 | 935/038 | 5329 |  | N.D. | B | B | B | B | B | B |  | MS |
| 84 | Styrene | 13.898 | 1239 | 1241 |  | 19.0 | 0.30 | 925/925 | 3546 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 85 | o-Cymene | 14.351 | 1255 | 1261 |  | 19.5 | 4.66 | 873/874 | 4480 |  | N.D. | N.D. | A | A+B | A+B | A | A |  | MS,RI,O |
| 86 | 1,2,4-Trimethylbenzene | 14.625 | 1265 | 1278 |  | 20.0 | 4.28 | 858/868 | 2342 |  | N.D. | B | B | A+B | N.D. | B | A |  | MS,RI |
| 87 | m-Cymene |  |  |  |  | 26.7 | 3.86 | 897/914 | 2764 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 88 | 1,3,8-p-Menthatriene |  |  |  |  | 26.7 | 3.92 | 851/869 | 1425 |  | N.D. | B | B | B | N.D. | B | B |  | MS |
| 89 | 1-Methylindan |  |  |  |  | 29.2 | 4.02 | 839/842 | 4658 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 90 | Naphthalene | 25.784 | 1719 | 1740 |  | 38.4 | 2.90 | 904/924 | 6100 |  | A+B | A | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 91 | Decane | 7.125 | 988 | 1000 |  |  |  |  |  |  | N.D. | A | A | A | A | A | A |  | MS,RI |
| 92 | Dodecane | 12.540 | 1190 | 1200 |  |  |  |  |  |  | N.D. | A | A | A | A | A | A |  | MS,RI,O |
| 93 | Tridecane | 15.291 | 1289 | 1293 |  |  |  |  |  |  | N.D. | A | A | A | A | A | A |  | MS,RI |
| 94 | Tetradecane | 17.930 | 1389 | 1400 |  |  |  |  |  |  | N.D. | N.D. | A | N.D. | A | A | A |  | MS,RI |
| 95 | 1,2,4,5-Tetramethylbenzene | 18.712 | 1419 | 1435 |  |  |  |  |  |  | A | A | A | N.D. | A | A | A |  | MS,RI |
| 96 | Pentadecane | 20.461 | 1489 | 1500 |  |  |  |  |  |  | N.D. | A | A | N.D. | N.D. | N.D. | N.D. |  | MS,RI |
| 97 | Longifolene | 22.211 | 1562 |  |  |  |  |  |  |  | N.D. | N.D. | N.D. | N.D. | A | A | A |  | MS |
|  | **Esters (13)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 98 | Ethyl acetate |  |  |  |  | 6.5 | 2.64 | 930/933 | 9285 |  | N.D. | B | B | B | B | B | B |  | MS |
| 99 | Ethenyl acetate |  |  |  |  | 6.6 | 2.32 | 930/935 | 6630 |  | N.D. | B | B | B | B | B | B |  | MS |
| 100 | Butyl butanoate |  |  |  |  | 17.4 | 5.06 | 870/871 | 4903 |  | N.D. | B | B | B | N.D. | B | B |  | MS |
| 101 | Isoamyl isobutyrate |  |  |  |  | 19.5 | 5.14 | 890/890 | 5765 |  | N.D. | B | B | B | B | B | B |  | MS |
| 102 | Hexyl acetate |  |  |  |  | 19.8 | 4.94 | 810/851 | 8386 |  | N.D. | N.D. | B | N.D. | B | N.D. | N.D. |  | MS |
| 103 | Hexyl butanoate |  |  |  |  | 26.0 | 4.94 | 850/878 | 7166 |  | N.D. | B | B | B | B | B | B |  | MS |
| 104 | 1-(Acetyloxy)-2-propanone |  |  |  |  | 27.6 | 2.16 | 929/933 | 7829 |  | N.D. | B | B | B | B | B | B |  | MS |
| 105 | Butyrolactone |  |  |  |  | 34.0 | 2.20 | 925/937 | 9063 |  | B | B | B | B | B | B | B |  | MS |
| 106 | 5-Ethyldihydro-2(3H)-furanone |  |  |  |  | 36.9 | 2.46 | 854/875 | 6660 |  | N.D. | B | B | N.D. | B | B | B |  | MS |
| 107 | Hexanolactone |  |  |  |  | 40.1 | 2.48 | 821/878 | 5374 |  | N.D. | N.D. | B | B | N.D. | N.D. | B |  | MS |
| 108 | Phenethyl acetate |  |  |  |  | 41.2 | 2.64 | 856/872 | 7724 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 109 | Eugenol acetate |  |  |  |  | 55.3 | 2.36 | 867/869 | 4599 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 110 | Coumarin |  |  |  |  | 60.4 | 2.30 | 807/865 | 8493 |  | N.D. | N.D. | N.D. | B | N.D.．． | B | N.D. |  | MS |
|  | **Ethers (5)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 111 | Anethole | 27.631 | 1804 | 1809 |  | 41.6 | 2.70 | 911/917 | 5606 |  | A+B | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 112 | Estragole | 24.232 | 1649 | 1655 |  | 41.7 | 2.74 | 872/887 | 5283 |  | N.D. | A | B | B | A+B | A+B | A+B |  | MS,RI,O |
| 113 | Methyleugenol | 31.297 | 1985 | 2006 |  | 47.8 | 2.58 | 863/868 | 9018 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI |
| 1147 | Elemicin |  |  |  |  | 54.5 | 2.48 | 834/838 | 8459 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 1158 | Myristicin | 35.781 | 2235 | 2257 |  | 55.5 | 2.42 | 849/850 | 9720 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI,O  ,O |
|  | **Phenols (3)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 116 | Phenol |  |  |  |  | 47.2 | 1.70 | 907/907 | 9075 |  | B | N.D. | N.D. | B | B | B | B |  | MS |
| 117 | Eugenol | 34.130 | 2136 | 2141 |  | 52.5 | 2.04 | 909/910 | 4759 |  | N.D. | N.D. | N.D. | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 118 | trans-Isoeugenol | 35.711 | 2227 | 2250 |  |  |  |  |  |  | N.D. | N.D. | N.D. | A | A | A | A |  | MS,RI,O |
|  | **Acids (4)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 119 | Acetic acid |  |  |  |  | 22.4 | 1.74 | 936/951 | 9794 |  | N.D. | N.D. | B | B | B | B | B |  | MS |
| 120 | Butanoic acid |  |  |  |  | 29.3 | 1.86 | 840/876 | 9013 |  | B | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |  | MS |
| 121 | Pentanoic acid |  |  |  |  | 36.6 | 1.94 | 881/900 | 8026 |  | B | N.D. | B | B | B | B | B |  | MS |
| 122 | Octanoic acid |  |  |  |  | 44.0 | 1.96 | 856/856 | 7727 |  | B | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |  | MS |
|  | **Furan, N- or S-containing compounds (17)** | | | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 123 | Methanethiol |  |  |  |  | 4.5 | 1.64 | 931/931 | 9863 |  | B | B | B | B | B | B | B |  | MS,O |
| 124 | Dimethyl disulfide |  |  |  |  | 11.5 | 4.76 | 867/878 | 9694 |  | N.D. | B | B | N.D. | N.D. | N.D. | N.D. |  | MS |
| 125 | 3-Methylthiophene |  |  |  |  | 12.1 | 3.00 | 902/902 | 5186 |  | N.D. | B | B | B | B | B | B |  | MS |
| 126 | Pyridine |  |  |  |  | 16.1 | 3.14 | 848/874 | 8676 |  | B | B | B | B | B | B | B |  | MS |
| 127 | 2-Pentylfuran | 13.276 | 1216 | 1231 |  | 17.8 | 3.94 | 880/880 | 6098 |  | N.D. | A+B | A+B | A+B | A+B | A+B | A+B |  | MS,RI,O |
| 128 | Dimethyl trisulfide |  |  |  |  | 24.2 | 3.40 | 839/852 | 9866 |  | N.D. | B | N.D. | N.D. | B | B | B |  | MS,O |
| 129 | 3-(4-Methyl-3-pentenyl)-furan |  |  |  |  | 26.0 | 3.60 | 809/819 | 7961 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 130 | Furfural |  |  |  |  | 27.5 | 2.12 | 915/924 | 9442 |  | N.D. | N.D. | B | B | B | B | B |  | MS |
| 131 | 2-Propylpyridine |  |  |  |  | 32.3 | 3.82 | 814/830 | 3931 |  | N.D. | N.D. | B | N.D. | N.D. | N.D. | N.D. |  | MS |
| 132 | 2-Acetylthiazole |  |  |  |  | 34.8 | 2.38 | 860/882 | 9841 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 133 | 2-Furanmethanol |  |  |  |  | 35.1 | 1.76 | 834/894 | 8184 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 134 | 2-Thiophenecarboxaldehyde |  |  |  |  | 36.5 | 2.30 | 850/863 | 6376 |  | N.D. | N.D. | B | B | N.D. | N.D. | N.D. |  | MS |
| 135 | Safrole |  |  |  |  | 43.2 | 2.62 | 856/861 | 6791 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 136 | Dimethyl sulfone |  |  |  |  | 43.6 | 1.82 | 823/870 | 9776 |  | B | N.D. | B | B | B | B | B |  | MS |
| 137 | Benzothiazole |  |  |  |  | 45.7 | 2.54 | 826/836 | 9670 |  | N.D. | B | B | N.D. | B | B | B |  | MS |
| 138 | 2-Acetylpyrrole |  |  |  |  | 46.1 | 1.88 | 831/863 | 9714 |  | N.D. | N.D. | N.D. | B | B | B | B |  | MS |
| 139 | 5-Hydroxymethylfurfural | 38.915 | 2435 | 2512 |  |  |  |  |  |  | N.D. | A | A | N.D. | N.D. | N.D. | N.D. |  | MS,RI,O |

***Note:*** FP, fresh pork; SP1, stewed pork with water; SP2: stewed pork in water and salt; SP3: stewed pork in water, salt and spices; SP4: stewed pork in water, salt, spices and soy sauce; SP5: stewed pork in water, salt, spices, soy sauce and sugar; SP6: stewed pork in water, salt, spices, soy sauce, sugar and cooking wine.

1 RI: retention index.

2 RI\*: retention indexes from <http://www.odour.org.uk/>.

3 Identification method: MS, mass spectrum comparison using NIST libraries; O, odor description.

4 B: Volatile compounds were detected by GC × GC-TOFMS.

5 N.D.: Volatile compounds were not detected by GC × GC-TOFMSor GC-MS/O.

6 A+B: Volatile compounds were detected by both GC × GC-TOFMS and GC-MS/O.

7 A: Volatile compounds were detected by GC-MS/O.

**Table 4 - 3:** The comparison of kinds and content ratios of volatile components by GC-MS/O and GC × GC-TOFMS.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | | GC-MS/O | | | | | | |  | | GC × GC-TOFMS | | | | | |
| FP | SP1 | SP2 | SP3 | SP4 | SP5 | SP6 |  | FP | SP1 | SP2 | SP3 | SP4 | SP5 | SP6 |
| Quantities | Aldehydes | 5 | 12 | 12 | 11 | 11 | 11 | 11 |  | 20 | 22 | 22 | 20 | 19 | 21 | 22 |
| Alcohols | 3 | 5 | 5 | 6 | 7 | 7 | 8 |  | 14 | 13 | 16 | 17 | 19 | 16 | 18 |
| Ketones | 0 | 0 | 0 | 3 | 3 | 3 | 3 |  | 8 | 10 | 12 | 13 | 11 | 14 | 13 |
| Hydrocarbons | 6 | 11 | 13 | 11 | 13 | 13 | 14 |  | 6 | 11 | 12 | 18 | 13 | 15 | 13 |
| Esters | 0 | 0 | 0 | 0 | 0 | 0 | 0 |  | 1 | 8 | 10 | 11 | 10 | 11 | 11 |
| Ethers | 1 | 2 | 1 | 3 | 4 | 4 | 4 |  | 1 | 1 | 2 | 5 | 5 | 5 | 5 |
| Phenols | 0 | 0 | 0 | 2 | 2 | 2 | 2 |  | 1 | 0 | 0 | 2 | 2 | 2 | 2 |
| Acids | 0 | 0 | 0 | 0 | 0 | 0 | 0 |  | 3 | 0 | 2 | 2 | 2 | 2 | 2 |
| Heterocyclic and sulfur compounds | 0 | 2 | 2 | 1 | 1 | 1 | 1 |  | 3 | 7 | 10 | 12 | 13 | 13 | 13 |
| Total | 15 | 32 | 33 | 37 | 41 | 41 | 43 |  | 57 | 72 | 86 | 100 | 94 | 99 | 99 |
| Ratios (%) | Aldehydes | 37.8 | 66.8 | 63.7 | 34.9 | 40.2 | 37.8 | 43.2 |  | 43.1 | 30.6 | 38.9 | 20.0 | 17.9 | 18.5 | 19.9 |
| Alcohols | 9.1 | 11.8 | 12.1 | 26.3 | 21.1 | 21.4 | 18.5 |  | 13.5 | 13.3 | 6.2 | 15.8 | 7.8 | 8.1 | 6.6 |
| Ketones | 0.0 | 0.0 | 0.0 | 6.0 | 3.6 | 4.6 | 3.2 |  | 23.5 | 9.0 | 16.5 | 11.1 | 11.4 | 7.3 | 11.8 |
| Hydrocarbons | 48.4 | 12.3 | 16.7 | 18.9 | 24.6 | 23.7 | 25.4 |  | 16.8 | 29.1 | 19.7 | 40.7 | 50.1 | 47.3 | 48.9 |
| Esters | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.1 | 2.8 | 5.2 | 2.5 | 3.7 | 9.1 | 4.4 |
| Ethers | 4.8 | 1.2 | 1.0 | 6.2 | 3.7 | 4.6 | 3.6 |  | 0.2 | 0.2 | 0.2 | 2.4 | 0.8 | 0.9 | 0.5 |
| Phenols | 0.0 | 0.0 | 0.0 | 2.9 | 3.8 | 4.5 | 2.7 |  | 0.01 | 0.0 | 0.0 | 0.5 | 0.5 | 0.7 | 0.2 |
| Acids | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 2.7 | 0.3 | 0.5 | 0.4 | 0.3 | 0.6 | 0.5 |
| Heterocyclic and sulfur compounds | 0.0 | 7.9 | 6.4 | 4.9 | 3.0 | 3.3 | 3.4 |  | 0.1 | 14.7 | 12.9 | 6.5 | 7.5 | 7.5 | 7.0 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |  | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

***Note:*** FP, fresh pork; SP1, stewed pork with water; SP2: stewed pork in water and salt; SP3: stewed pork in water, salt and spices; SP4: stewed pork in water, salt, spices and soy sauce; SP5: stewed pork in water, salt, spices, soy sauce and sugar; SP6: stewed pork in water, salt, spices, soy sauce, sugar and cooking wine.

## *3.2. Volatile compounds profiling in the fresh and stewed pork*

A total of 139 volatile compounds of the fresh and stewed pork were extracted and identified by GC-MS/O and GC × GC-TOFMS, including 30 aldehydes, 23 alcohols, 19 ketones, 25 hydrocarbons, 13 esters, 5 ethers, 3 phenols, 4 acids, 17 heterocyclic and sulphur-containing compounds (Table 4 - 4). Most of these compounds have been reported in several pork products (Lorenzo & Fonseca, 2014; Yang, Sun, Pan, Wang, & Cao, 2018). Aldehydes are mainly derived from the lipid oxidation and degradation reaction, and Strecker degradation of amino acids (Han et al., 2019; Zou et al., 2018), as they have lower odour thresholds which are considered the major contributions to the overall flavour of meat products (Li et al., 2016; Petričević, Radovčić, Lukić, Listeš, & Medić, 2018). During the seven different pork samples, the content of aldehydes was the most abundant, with the mean total amounts of 619.8-3109.6 μg·kg-1. Obviously, the content of aldehydes in heated pork samples (SP1, SP2, SP3, SP4, SP5 and SP6) were significantly greater (*P < 0.001*) than those in the fresh pork. This indicated that the thermal processing of pork products plays a crucial role in the formation of aldehydes (Aaslyng & Meinert, 2017). It can be also found that 15 compounds, namely, 9 saturated aldehydes (butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal and pentadecanal and 9-octadecenal), 4 olefin aldehydes ((*E*)-2-octenal, (*E*)-2-nonenal, cis-4-decenal and (*E*)-2-decenal), as well as 2 aromatic aldehydes (benzaldehyde and benzeneacetaldehyde) were simultaneously identified in all pork samples. The saturated and olefin aldehydes could be produced from the degradation of fatty acids (Jayasena, Ahn, Nam, & Jo, 2013) and the aromatic aldehydes were usually generated from Strecker reaction (Gu, Wang, Tao, & Wu, 2013). Among them, except for pentanal and benzeneacetaldehyde, the contents of other 13 compounds were significantly higher (*P < 0.001*) in SP1 and SP2 than those in SP3, SP4, SP5 and SP6. Becides, (Z)-2-heptenal, 2-butyl-a-octenal and tertradecanal were detected only in SP1 and SP2. It showed that the spices, soy sauce, sugar and cooking wine may had an inhibitory effect on the production of some aldehydes in stewed pork. Moreover, 4-(1-methylethyl)-benzaldehyde, p-anisaldehyde and cinnamaldehyde, with mint and sweet aroma (Li et al., 2016), could be generated from the Chinese different spices including fennel, aniseed and cinnamon respectively, since they were all identified when spices were involved in stewed pork (TM3, TM4, TM5 and TM6).

Alcohols, with pleasant fruity and floral notes (Wang et al., 2018), are mainly derived from spices (Gong et al., 2017) and the oxidative decomposition of lipid (Toldrá, 2017). For the identified alcohols, 1,8-cineole, 1-pentanol, 1-hexanol, 1-octen-3-ol, 1-heptanol, 2-etyl-1-hexanol, 1-octanol and (*E*)-2-octen-1-ol could be detected in all pork samples. Of which the linear alcohols (1-pentanol, 1-hexanol, 1-heptanol, 1-octanol and (*E*)-2-octen-1-ol) and branched alcohols (1-octen-3-ol and 2-etyl-1-hexanol) were the main lipid oxidation products. Meanwhile, the content of these compounds was significantly higher (*P < 0.01*) in SP1 and SP2 than those in FP, SP2, SP3, SP4, SP5 and SP6. The 1,8-cineole, linalool, terpinen-4-ol, terpineol, (4R,6R)-2-undecen-1-ol and cis-geraniol increased significantly (*P < 0.001*) in SP3 as the spices were added in the stewed pork and greatly reduced in SP4, SP5 and SP6. This result showed that they were mainly formed by spiced brine (spices, soy sauce, sugar and cooking wine).

Ketones were often considered to have a great influence on the aroma of meat and meat products since they were presented in large amounts and exhibited specific aroma in food (Van Ba, Hwang, Jeong, & Touseef, 2012). As shown in Table 4.4, 7 ketones such as 6-methyl-5-hepten-2-one, 4-isopropyl-2-cyclohexenone, piperitone, carvone, 4-pehenyl-2-butanone, 4-methoxyphenylacetone and xanthoxylin were detected in the spice, soy sauce, sugar and cooking wine processed samples (SP3, SP4, SP5 and SP6), and no ketone was detected in fresh pork (FP) and stewed pork with water and salt (SP1 and SP2). It indicated that more ketone compounds were formed due to the addition of food seasoning, which provided a richer fruity and nutty aroma (Lorenzo & Fonseca, 2014) for the overall pork flavour. Pham et al., (2008) reported that the methyl ketones were considered as the precursors to the fatty aromas related to cooked meat, which could be formed the oxidative degradation of fatty acids (José M Lorenzo, Montes, Purriños, & Franco, 2012). In this study, the methyl ketones (e.g. 2-heptanone, 2-octanone, 2,3-pentanedione, 2,3-octanedione, 3-octen-2-one, (*E,E*)-3,5-octadien-2-one, 6-methyl-3,5-heptadiene-2-one and acetophenone) were originated from the oxidation of lipids during heating. The results were relatively agreed with those found by (Wang et al., 2018).

There were very few hydrocarbons detected in the fresh pork, however more hydrocarbons could be formed by added water, salt, soy sauce, sugar and cooking wine during the pork processing. All hydrocarbons could be divided into aromatic hydrocarbons and aliphatic hydrocarbons. Among them, 16 aromatic hydrocarbons had been identified in all stewed pork samples and toluene, ethylbenzene, 1,3-dimethylbenzene, o-xylene and styrene were present as common compounds. The production of toluene and ethylbenzene primarily come from amino acid degradation. This result was consistent with the reported by (Olivares et al., 2011). It was also found that 9 aliphatic hydrocarbons were detected in stewed pork samples (SP1, SP2, SP3, SP4, SP5 and SP6). Previous study has shown that aliphatic hydrocarbons had a limited influence on aroma perception due to their high threshold values (Qi, Liu, Zhou, & Xu, 2017) and raised mainly from lipid oxidation (Petričević et al., 2018). Additionally, phellandrene, (Z)-3,7-dimethyl-1,3,6-octatriene, o-cymene, m-cymene, 1-methylindan and longifolene could be formed from the added spices and soy sauce during the processing.

Ester compounds could be formed by the esterification of alcohols and carboxylic acid in the meat products (Han et al., 2019). The contribution of the esters to the aroma of pork products depends on the length of their chain (Petričević et al., 2018). A total of 13 esters were identified in all stewed pork samples, where short-chain esters, such as ethyl acetate and ethenyl acetate had fruity notes. While long-chain esters like isoamyl isobutyrate, hexyl butanoate, hexyl acetate and hexyl butanoate possessed a slight fatty odour (Ramírez & Cava, 2007). In addition, when the salt was added in SP2, the relative content of esters was significantly increased (*P < 0.05*). This reason might be that the salt of meat products favoured the formation of ester compounds. For ether and phenol compounds, the anethole was detected in all pork samples and the methyleugenol, elemicin, myristicin, eugenol and trans-isoeugenol were found in SP3, SP4, SP5 and SP6. Moreover, the ethers and phenols except for phenol were from the spices and the acids (butanoic acid, pentanoic acid and octanoic acid) come from the fresh pork.

**Table 4 - 4:** Concentrations and origin of volatile compounds of the fresh and stewed pork.

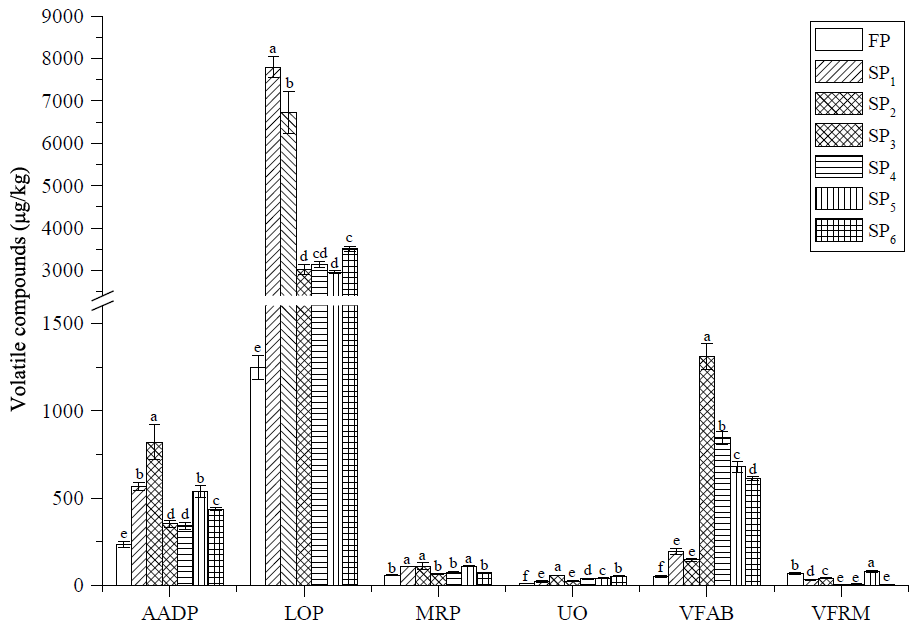
|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Compounds | FP | SP1 | SP2 | SP3 | SP4 | SP5 | SP6 | *p* value | Origin1 |
|  | **Aldehydes (30)** | 719.6±36.8d | 2478.5±104.6b | 3326.1±219.3a | 959.1±1.9c | 795.9±7.8cd | 815.1±25.8cd | 934.2±44.1c | 0.000 |  |
| 1 | Acetaldehyde | 1.7±0.2a | N.D. | N.D. | N.D. | N.D. | 1.7±0.3a | 0.9±0.1b | 0.011 | AADP |
| 2 | Propanal | N.D. | N.D. | 97.9±15.0a | N.D. | 16.1±3.2b | 25.5±1.7b | 29.3±2.4b | 0.000 | AADP |
| 3 | Butanal | 0.5±0.2d | 3.7±0.8b | 19.0±1.5a | 2.1±0.9c | 3.5±0.6b | 2.0±0.3c | 2.0±0.3c | 0.000 | LOP |
| 4 | Pentanal | 27.5±4.0e | 53.6±6.9d | 74.3±1.3ab | 80.7±3.8a | 60.4±9.8cd | 70.0±5.6bc | 65.7±3.7bc | 0.000 | LOP |
| 5 | Hexanal | 3.5±1.0e | 633.2±13.3a | 634.1±14.7a | 233.2±8.9c | 253.8±10.1b | 176.4±10.5d | 221.2±15.4c | 0.000 | LOP |
| 6 | Heptanal | 56.7±3.4c | 122.6±15.7b | 160.1±2.1a | 31.5±1.7d | 36.9±1.2d | 38.3±3.6d | 38.2±7.2d | 0.000 | LOP |
| 7 | Octanal | 84.7±10.9c | 182.0±10.3b | 230.9±25.1a | 62.4±5.0d | 86.4±9.7c | 50.5±4.3d | 54.9±8.6d | 0.000 | LOP |
| 8 | (*Z*)-2-Heptenal | N.D. | 5.6±0.2b | 38.5±9.0a | N.D. | N.D. | N.D. | N.D. | 0.024 | LOP |
| 9 | 2-Methylpentanal | N.D. | 3.6±0.1a | N.D. | 1.7±0.3b | N.D. | N.D. | 1.7±0.01b | 0.000 | LOP |
| 10 | Nonanal | 219.8±13.3cd | 1475.5±124.6a | 844.4±94.8b | 316.3±5.8c | 171.4±17.8d | 110.3±7.9d | 284.2±11.9c | 0.000 | LOP |
| 11 | (*E*)-2-Octenal | 24.6±1.1c | 76.0±1.2a | 42.2±7.4b | 3.2±0.2d | 5.5±1.1d | 6.6±1.8d | 8.3±0.6d | 0.000 | LOP |
| 12 | Decanal | 5.2±0.6bc | 9.1±1.8a | 8.0±0.7a | 4.4±0.2bc | 4.5±0.6bc | 4.2±0.2c | 5.9±0.3b | 0.001 | LOP |
| 13 | Benzaldehyde | 194.4±17.0c | 315.1±13.5b | 370.6±52.7a | 130.4±12.0de | 110.0±8.5e | 200.4±23.3c | 156.7±11.5cd | 0.000 | AADP |
| 14 | (*E*)-2-Nonenal | 7.5±0.9c | 11.3±2.5b | 15.1±1.0a | 1.6±0.1d | 1.8±0.7d | 2.4±0.4d | 1.9±0.4d | 0.000 | LOP |
| 15 | cis-4-Decenal | 11.9±1.0a | 14.1±2.8a | 14.2±2.3a | 3.4±0.3b | 3.6±0.4b | 4.0±0.4b | 3.0±0.1b | 0.000 | LOP |
| 16 | Benzeneacetaldehyde | 2.3±0.1d | 4.2±0.9c | 5.8±1.6be | 3.6±0.2cd | 7.4±0.4b | 9.5±1.3a | 6.5±0.4b | 0.000 | MRP |
| 17 | (*E*)-2-Decenal | 2.9±0.2b | 5.6±1.1a | 4.6±1.0a | 1.0±0.1c | 0.6±0.02c | 0.7±0.1c | 0.8±0.1c | 0.000 | LOP |
| 18 | 2-Butyl-2-octenal | N.D. | 6.3±0.3b | 10.1±1.4a | N.D. | N.D. | N.D. | N.D. | 0.038 | LOP |
| 19 | (*E,E*)-2,4-Nonadienal | 2.7±0.3b | 6.0±0.3a | 5.8±0.2a | N.D. | N.D. | N.D. | N.D. | 0.000 | LOP |
| 20 | 2-Undecenal | 2.5±0.2a | 2.7±0.7a | 2.4±0.3a | N.D. | N.D. | N.D. | N.D. | 0.717 | LOP |
| 21 | 4-(1-Methylethyl)-benzaldehyde | N.D. | N.D. | N.D. | 1.2±0.1a | 0.7±0.2c | 0.9±0.1b | 0.5±0.1c | 0.001 | VFAB |
| 22 | (*E,E*)-2,4-Decadienal | 7.3±1.0b | 10.0±0.5a | 7.9±0.9b | 0.8±0.01c | N.D. | 0.9±0.2c | 1.6±0.1c | 0.000 | LOP |
| 23 | p-Anisaldehyde | N.D. | N.D. | N.D. | 3.0±0.2b | 1.4±0.2d | 3.5±0.2a | 2.4±0.4c | 0.000 | VFAB |
| 24 | Cinnamaldehyde | N.D. | N.D. | N.D. | 2.0±0.4ab | 1.1±0.2b | 2.5±0.2a | 2.3±0.8a | 0.031 | VFAB |
| 25 | Pentadecanal | 33.9±0.6c | 127.9±5.2a | 66.2±11.4b | 32.4±1.5c | 18.4±3.3d | 79.8±7.5b | 28.7±4.5c | 0.000 | LOP |
| 26 | 10-Undecenal | 0.6±0.1b | 0.3±0.1c | 1.7±0.2a | N.D. | N.D. | N.D. | N.D. | 0.000 | LOP |
| 27 | 9-Octadecenal | 0.5±0.01d | 4.9±0.5a | 1.7±0.1b | 1.1±0.1c | 1.1±0.1c | 1.9±0.1b | 1.2±0.4c | 0.000 | LOP |
| 28 | β-Cyclocitral | 1.1±0.02b | N.D. | N.D. | N.D. | 4.2±1.2a | 3.5±0.2a | 1.9±0.2b | 0.001 | LOP |
| 29 | Tetradecanal | N.D. | 5.2±0.8a | 4.8±0.5a | 2.7±0.1b | N.D. | N.D. | N.D. | 0.003 | LOP |
| 30 | Hexadecanal | N.D. | 31.1±0.8c | 34.7±0.2b | 40.4±0.8a | 7.2±0.3f | 19.6±1.1d | 14.4±0.6e | 0.000 | LOP |
|  | **Alcohols (23)** | 224.8±14.1f | 1073.6±22.0a | 525.9±29.8c | 756.6±10.2b | 347.5±14.7d | 358.1±1.0d | 310.2±6.6e | 0.000 |  |
| 31 | 4-Methyl-1-pentanol | N.D. | N.D. | 3.0±0.2b | N.D. | N.D. | 6.2±0.2a | 2.3±0.1c | 0.000 | UO |
| 32 | 1-Penten-3-ol | N.D. | 17.1±2.7a | 16.2±2.7a | N.D. | 2.9±0.4c | N.D. | N.D. | 0.000 | LOP |
| 33 | 1-Butanol | 1.5±0.2c | N.D. | 5.6±1.1a | 1.7±0.1bc | 1.8±0.2bc | 2.5±0.5bc | 2.5±0.3b | 0.000 | LOP |
| 34 | 1,8-Cineole | 1.7±0.8e | 9.6±0.8d | 15.2±1.8d | 123.2±11.5a | 63.6±1.4b | 66.5±0.4b | 43.0±1.1c | 0.000 | VFAB |
| 35 | 1-Pentanol | 32.2±4.6c | 268.2±17.6a | 165.8±13.9b | 26.6±5.4c | 26.3±3.3c | 29.2±3.7c | 28.6±0.4c | 0.000 | LOP |
| 36 | (*Z*)-2-Penten-1-ol | N.D. | 1.3±0.3a | 1.5±0.1a | N.D. | N.D. | N.D. | N.D. | 0.312 | LOP |
| 37 | 1-Hexanol | 85.4±3.5b | 477.1±18.3a | 41.3±2.5c | 2.8±0.6e | 6.1±0.7e | 24.3±2.6d | 6.6±0.5e | 0.000 | LOP |
| 38 | 1-Octen-3-ol | 1.5±0.4g | 130.0±4.3b | 137.0±3.1a | 50.6±0.5d | 59.4±0.4c | 27.7±2.0f | 40.2±3.7e | 0.000 | LOP |
| 39 | 1-Heptanol | 10.3±1.6c | 31.6±4.9a | 24.9±3.0b | 3.3±0.4d | 4.8±3.6d | 2.5±0.2d | 3.7±0.2d | 0.000 | LOP |
| 40 | 2-Ethyl-1-hexanol | 8.7±2.3b | 8.1±1.3b | 11.6±0.9a | 5.1±0.2c | 5.6±0.3c | 7.9±0.3b | 5.3±0.8c | 0.000 | LOP |
| 41 | Linalool | N.D. | N.D. | N.D. | 75.6±5.9a | 23.0±2.0b | 22.3±0.9b | 21.8±2.7b | 0.000 | VFAB |
| 42 | 1-Octanol | 24.6±0.4c | 49.4±5.8a | 33.9±3.3b | 9.8±0.4d | 5.9±0.7d | 8.0±0.9d | 7.5±0.2d | 0.000 | LOP |
| 43 | 2,3-Butanediol | N.D. | N.D. | 4.0±0.7b | N.D. | 6.8±0.7a | N.D. | N.D. | 0.007 | LOP |
| 44 | Terpinen-4-ol | N.D. | N.D. | N.D. | 313.3±5.9a | 93.2±6.8b | 87.9±4.0b | 71.7±3.5c | 0.000 | VFAB |
| 45 | (*E*)-2-Octen-1-ol | 41.8±6.2c | 73.7±11.9a | 59.3±10.0b | 13.8±1.4d | 13.8±1.7d | 14.4±2.0d | 12.8±0.4d | 0.001 | LOP |
| 46 | 1-Nonanol | 6.3±0.8a | 1.7±0.2b | 0.6±0.01c | 0.3±0.1c | 0.2±0.01c | N.D. | 0.2±0.1c | 0.000 | LOP |
| 47 | Terpineol | N.D. | N.D. | N.D. | 113.2±3.5a | 21.0±2.1d | 52.5±1.9b | 45.4±1.4c | 0.000 | VFAB |
| 48 | trans-2-Undecen-1-ol | 4.8±0.8ab | 5.5±0.6a | 5.2±1.3ab | 2.9±0.2cd | 3.8±0.5bc | N.D. | 2.2±1.1d | 0.002 | LOP |
| 49 | (4R,6R)-cis-Carveol | N.D. | N.D. | N.D. | 0.8±0.02a | 0.5±0.1bc | 0.6±0.1b | 0.4±0.01c | 0.001 | VFAB |
| 50 | cis-Geraniol | N.D. | N.D. | N.D. | 11.3±1.7a | 7.4±0.6b | 3.4±0.1c | 7.7±0.8b | 0.000 | VFAB |
| 51 | Phenylethyl alcohol | 2.7±0.2b | N.D. | 0.8±0.02d | 2.3±0.1b | 1.4±0.3c | 2.2±0.1b | 8.3±0.5a | 0.000 | UO |
| 52 | 1-Undecanol | 3.0±0.4 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | VFRM |
| 53 | 1-Dodecanol | 0.3±0.01a | 0.3±0.01a | N.D. | N.D. | N.D. | N.D. | N.D. | 0.467 | VFRM |
|  | **Ketones (19)** | 377.2±17.6de | 731.7±42.2b | 1411.9±198.7a | 530.8±16.6cd | 506.6±7.2cd | 323.6±1.2e | 555.9±16.2c | 0.000 |  |
| 54 | 2-Butanone | 1.3±0.3d | 2.7±0.9d | 4.0±0.7cd | 30.5±4.9b | 35.0±7.0b | 9.3±0.7c | 49.0±2.9a | 0.000 | VFAB |
| 55 | 2,3-Butanedione | 12.5±2.0bc | 23.6±1.7a | 23.3±3.0a | 24.7±2.1a | N.D. | 15.0±1.3b | 11.1±0.5c | 0.000 | MRP |
| 56 | 2,3-Pentanedione | N.D. | 15.3±2.9a | 18.0±2.6a | N.D. | N.D. | 4.2±0.6c | 10.1±0.4b | 0.000 | LOP |
| 57 | 2-Heptanone | 25.2±2.6d | 60.3±8.5c | 118.5±11.2a | 19.4±1.7d | N.D. | 57.3±4.1c | 72.1±5.8b | 0.000 | LOP |
| 58 | 3-Octanone | 20.8±1.0a | 8.3±2.5c | 12.0±1.1b | N.D. | 2.1±0.4d | 6.0±1.2c | N.D. | 0.000 | VFRM |
| 59 | 2-Octanone | 7.2±1.7c | 12.0±3.1b | 18.3±0.6a | N.D. | N.D. | N.D. | N.D. | 0.002 | LOP |
| 60 | 1-Hydroxy-2-propanone | N.D. | 20.7±3.6d | 57.7±15.0b | 29.7±1.7d | 54.7±6.7bc | 73.2±2.2a | 43.0±2.2c | 0.000 | MRP |
| 61 | 2,3-Octanedione | 303.7±20.8c | 578.6±26.4b | 1122.0±184.4a | 321.4±9.1c | 339.3±7.4c | N.D. | 297.9±16.0c | 0.000 | LOP |
| 62 | 6-Methyl-5-hepten-2-one | N.D. | N.D. | N.D. | 3.8±0.5c | 5.2±1.0a | 4.0±0.6bc | 2.9±0.1c | 0.019 | VFAB |
| 63 | 3-Octen-2-one | 4.3±0.6c | 6.0±0.5b | 10.2±0.6a | N.D. | N.D. | N.D. | N.D. | 0.000 | LOP |
| 64 | (*E,E*)-3,5-Octadien-2-one | N.D. | N.D. | 24.1±3.1 | N.D. | N.D. | N.D. | N.D. | N.D. | LOP |
| 65 | 6-Methyl-3,5-heptadiene-2-one | N.D. | N.D. | 0.9±0.2a | N.D. | N.D. | 0.3±0.01b | N.D. | 0.003 | LOP |
| 66 | Acetophenone | 2.2±0.2bc | 4.2±0.9a | 2.9±0.3b | 2.6±0.2bc | 2.0±0.1c | 2.7±0.5bc | 2.3±0.2bc | 0.001 | LOP |
| 67 | 4-Isopropyl-2-cyclohexenone | N.D. | N.D. | N.D. | 2.0±0.1a | 1.2±0.1b | 2.1±0.3a | 1.4±0.2b | 0.002 | VFAB |
| 68 | Piperitone | N.D. | N.D. | N.D. | 54.5±2.9a | 38.6±4.2b | 41.5±3.3b | 37.5±1.8b | 0.001 | VFAB |
| 69 | Carvone | N.D. | N.D. | N.D. | 2.7±0.3a | 1.6±0.2b | N.D. | N.D. | 0.005 | VFAB |
| 70 | 4-Phenyl-2-butanone | N.D. | N.D. | N.D. | 1.9±0.1a | N.D. | 1.3±0.1c | 1.6±0.1b | 0.001 | VFAB |
| 71 | 4-Methoxyphenylacetone | N.D. | N.D. | N.D. | 0.7±0.02a | 0.4±0.1b | 0.8±0.1a | 0.4±0.0b | 0.006 | VFAB |
| 72 | Xanthoxylin | N.D. | N.D. | N.D. | 36.9±2.3a | 26.5±3.5b | 39.8±5.4a | 26.6±2.2b | 0.003 | VFAB |
|  | **Hydrocarbons (25)** | 280.1±26.5f | 2357.7±80.2a | 1680.4±41.0e | 1946.6±146.6d | 2227.3±43.0b | 2087.4±43.4c | 2295.6±30.1ab | 0.000 |  |
| 73 | 2-Methylbutane | N.D. | 2.9±0.6a | N.D. | 1.6±0.2b | N.D. | 3.1±0.2a | 2.7±0.3a | 0.005 | LOP |
| 74 | Benzene | N.D. | N.D. | 5.4±0.7a | 2.8±0.1b | N.D. | N.D. | N.D. | 0.002 | LOP |
| 75 | Toluene | 10.3±1.5e | 179.8±8.4b | 238.8±23.4a | 119.9±14.6d | 141.4±9.9cd | 226.7±15.0a | 163.3±8.8bc | 0.000 | AADP |
| 76 | Ethylbenzene | 27.3±0.8b | 11.3±0.4d | 16.3±1.5c | 48.3±5.9a | 5.2±1.8e | 1.2±0.2e | 3.6±0.06e | 0.000 | AADP |
| 77 | 1,3-Dimethylbenzene | 22.2±2.0d | 160.0±7.1b | 140.2±30.3b | 148.7±15.1b | 295.2±9.6a | 86.2±13.8c | 149.1±10.6b | 0.000 | AADP |
| 78 | p-Xylene | N.D. | 270.1±4.8d | 367.3±10.0c | 483.5±80.8b | 525.5±21.4b | 618.1±10.2a | 622.4±18.3a | 0.000 | LOP |
| 79 | o-Xylene | 168.3±20.3d | 992.9±77.6a | 221.5±11.9cd | 241.5±19.1c | 85.1±3.5e | 250.7±17.4c | 474.0±17.9b | 0.000 | LOP |
| 80 | D-Limonene | N.D. | 12.5±1.8c | 22.0±0.7b | 13.2±1.7c | 21.8±2.4b | 29.9±3.1a | 13.3±0.7c | 0.000 | VFAB |
| 81 | Phellandrene | N.D. | N.D. | N.D. | 3.4±1.1a | 2.3±0.2a | N.D. | N.D. | 0.218 | VFAB |
| 82 | (Z)-3,7-Dimethyl-1,3,6-octatriene | N.D. | N.D. | N.D. | 10.9±0.8b | N.D. | 13.4±0.8a | N.D. | 0.019 | VFAB |
| 83 | 1,3,5,7-Cyclooctatetraene | N.D. | 456.8±15.0d | 435.3±17.3d | 445.6±11.5d | 621.5±16.7a | 567.1±22.7b | 530.5±17.1c | 0.000 | LOP |
| 84 | Styrene | 45.0±6.3d | 151.2±17.9b | 88.2±11.2cd | 323.8±72.7a | 328.1±32.5a | 113.3±9.2bc | 104.8±7.9bc | 0.000 | VFAB |
| 85 | o-Cymene | N.D. | N.D. | N.D. | 27.3±2.0a | 20.6±4.4a | N.D. | N.D. | 0.101 | VFAB |
| 86 | 1,2,4-Trimethylbenzene | N.D. | 10.5±0.5a | 10.9±1.0a | 8.4±0.7b | N.D. | 7.5±1.4b | N.D. | 0.007 | LOP |
| 87 | m-Cymene | N.D. | N.D. | N.D. | 0.4±0.1d | 77.2±4.0c | 93.6±7.5b | 128.9±13.4a | 0.000 | VFAB |
| 88 | 1,3,8-p-Menthatriene | N.D. | 22.9±2.2a | 6.2±0.5c | 6.2±1.0c | N.D. | 9.2±1.0b | 7.9±0.8bc | 0.000 | LOP |
| 89 | 1-Methylindan | N.D. | N.D. | N.D. | 7.5±0.4a | 7.7±0.3a | 6.9±0.3a | 7.5±1.7a | 0.123 | VFAB |
| 90 | Naphthalene | 3.5±0.4c | N.D. | 6.8±0.4b | 7.8±1.1ab | 7.2±0.5ab | 7.9±0.4ab | 8.2±0.7a | 0.000 | UO |
| 91 | Decane | N.D. | 6.4±0.3bc | 9.3±0.2a | 7.2±0.7bc | 7.5±1.3b | 5.9±1.0c | 6.3±0.3bc | 0.002 | LOP |
| 92 | Dodecane | N.D. | 26.3±0.8c | 34.9±1.8a | 31.2±0.9b | 34.2±2.0a | 19.6±0.9d | 29.6±1.3b | 0.000 | LOP |
| 93 | Tridecane | N.D. | 20.4±1.6a | 16.2±2.1b | 7.4±1.2d | 9.6±0.2c | 5.9±0.1d | 6.3±0.1d | 0.000 | LOP |
| 94 | Tetradecane | N.D. | N.D. | 21.4±1.0a | N.D. | 12.9±1.3b | 9.3±0.4c | 14.0±0.6b | 0.000 | LOP |
| 95 | 1,2,4,5-Tetramethylbenzene | 3.5±0.4e | 24.4±3.2ab | 27.0±1.6a | N.D. | 21.7±1.8b | 8.4±0.2d | 17.4±1.2c | 0.000 | UO |
| 96 | Pentadecane | N.D. | 9.3±0.3b | 12.7±0.9a | N.D. | N.D. | N.D. | N.D. | 0.003 | LOP |
| 97 | Longifolene | N.D. | N.D. | N.D. | N.D. | 2.6±0.05b | 3.5±1.4b | 5.8±0.6a | 0.011 | VFAB |
|  | **Esters (13)** | 1.9±0.3f | 222.8±19.4c | 440.8±24.6a | 120.8±14.0e | 166.2±12.6d | 399.9±12.9b | 205.8±12.0c | 0.000 |  |
| 98 | Ethyl acetate | N.D. | 46.7±3.9c | 69.9±3.8a | 35.6±7.3d | 58.8±1.2b | 34.6±4.2d | 47.8±1.0c | 0.000 | LOP |
| 99 | Ethenyl acetate | N.D. | 60.9±7.6c | 210.3±21.3b | 26.8±3.1d | 2.4±0.8e | 247.0±11.9a | 27.0±2.7d | 0.000 | LOP |
| 100 | Butyl butanoate | N.D. | 8.2±0.6c | 12.3±0.5b | 3.3±0.6d | N.D. | 15.0±1.2a | 14.2±1.2a | 0.022 | LOP |
| 101 | Isoamyl isobutyrate | N.D. | 59.8±8.3c | 82.7±5.3a | 21.4±2.9d | 69.5±9.4bc | 69.2±4.5bc | 78.1±7.4ab | 0.000 | LOP |
| 102 | Hexyl acetate | N.D. | N.D. | 3.7±0.3b | N.D. | 2.5±0.2a | N.D. | N.D. | 0.004 | UO |
| 103 | Hexyl butanoate | N.D. | 2.4±0.3b | 3.7±0.2a | 0.9±0.2d | 1.5±0.4c | 1.4±0.3c | 3.2±0.2a | 0.000 | LOP |
| 104 | 1-(Acetyloxy)-2-propanone | N.D. | 40.9±2.7b | 52.2±7.0a | 25.2±1.4c | 23.9±2.3c | 23.8±1.9c | 29.8±3.2c | 0.000 | LOP |
| 105 | Butyrolactone | 1.9±0.3d | 2.1±0.5bc | 3.9±0.6a | 1.7±0.2d | 2.6±0.5b | 2.1±0.2bc | 2.4±0.1b | 0.000 | LOP |
| 106 | 5-Ethyldihydro-2(3H)-furanone | N.D. | 1.8±0.4b | 2.0±0.3a | N.D. | 0.5±0.1c | 0.6±0.01c | 0.5±0.1c | 0.000 | LOP |
| 107 | Hexanolactone | N.D. | N.D. | 0.1±0.01b | 0.8±0.2a | N.D. | N.D. | 0.9±0.1a | 0.000 | LOP |
| 108 | Phenethyl acetate | N.D. | N.D. | N.D. | 1.9±0.1a | 0.8±0.2b | 0.8±0.2b | 0.6±0.1b | 0.000 | VFAB |
| 109 | Eugenol acetate | N.D. | N.D. | N.D. | 3.0±0.5b | 3.7±0.6b | 5.1±0.5a | 1.3±0.1c | 0.000 | VFAB |
| 110 | Coumarin | N.D. | N.D. | N.D. | 0.2±0.01b | N.D. | 0.3±0.01a | N.D. | 0.041 | VFAB |
|  | **Ethers (5)** | 2.8±0.5f | 16.5±0.5e | 16.9±2.1e | 116.8±3.2a | 37.1±2.8c | 41.7±2.2b | 25.0±2.0d | 0.000 |  |
| 111 | Anethole | 2.8±0.5e | 16.5±0.5d | 16.6±2.1d | 80.7±1.7a | 33.3±2.9b | 35.1±1.8b | 21.8±1.8c | 0.000 | VFAB |
| 112 | Estragole | N.D. | N.D. | 0.3±0.1c | 31.5±1.6a | 1.5±0.2bc | 2.9±0.3b | 2.1±0.5b | 0.000 | VFAB |
| 113 | Methyleugenol | N.D. | N.D. | N.D. | 2.0±0.1a | 0.9±0.2c | 1.3±0.1b | 0.4±0.01d | 0.000 | VFAB |
| 114 | Elemicin | N.D. | N.D. | N.D. | 1.1±0.2a | 0.6±0.1b | 1.3±0.1a | 0.4±0.01b | 0.000 | VFAB |
| 115 | Myristicin | N.D. | N.D. | N.D. | 1.5±0.2a | 0.8±0.1c | 1.1±0.04b | 0.3±0.02d | 0.000 | VFAB |
|  | **Phenols (3)** | 0.2±0.01e | N.D. | N.D. | 25.4±2.3b | 21.0±0.6c | 31.7±4.5a | 10.6±0.3d | 0.000 |  |
| 116 | Phenol | 0.2±0.01a | N.D. | N.D. | 0.2±0.02a | 0.2±0.02a | 0.2±0.01a | 0.2±0.02a | 0.121 | UO |
| 117 | Eugenol | N.D. | N.D. | N.D. | 21.6±2.8b | 18.4±0.7b | 28.9±4.5a | 9.2±0.5c | 0.000 | VFAB |
| 118 | trans-Isoeugenol | N.D. | N.D. | N.D. | 3.6±0.6a | 2.4±0.2b | 2.6±0.05b | 1.8±0.3c | 0.000 | VFAB |
|  | **Acids (4)** | 45.2±3.2a | 23.0±1.3bc | 45.0±2.9a | 19.9±1.4c | 13.6±0.9d | 24.9±2.1b | 24.2±0.6b | 0.000 |  |
| 119 | Acetic acid | N.D. | N.D. | 15.6±1.4b | 14.7±1.7b | 6.9±0.5c | 18.6±1.9a | 16.6±1.4ab | 0.000 | UO |
| 120 | Butanoic acid | 10.5±1.7 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | VFRM |
| 121 | Pentanoic acid | 32.1±2.0a | N.D. | 29.4±2.0a | 5.2±0.3c | 6.7±0.7b | 6.3±0.4b | 7.6±1.2b | 0.000 | VFRM |
| 122 | Octanoic acid | 2.6±0.3 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | VFRM |
|  | **Heterocyclic and sulfur compounds (17)** | 2.4±0.2c | 1192.7±104.0a | 1099.0±198.8a | 312.4±32.1b | 331.9±17.2b | 331.8±2.3b | 330.8±19.7b | 0.000 |  |
| 123 | 2-Pentylfuran | N.D. | 1071.5±108.9a | 975.5±178.1a | 249.0±28.0b | 250.1±21.7b | 234.3±3.1b | 234.2±15.9b | 0.001 | LOP |
| 124 | 3-(4-Methyl-3-pentenyl)-furan | N.D. | N.D. | N.D. | 4.7±1.0b | 3.6±0.03c | 5.8±0.1a | 1.7±0.1d | 0.000 | MRP |
| 125 | Furfural | N.D. | N.D. | 1.9±0.4b | 2.1±0.3b | 2.9±0.2a | 3.0±0.7a | 3.4±0.2a | 0.005 | MRP |
| 126 | 2-Furanmethanol | N.D. | N.D. | N.D. | 0.2±0.1c | 0.5±0.1b | 0.7±0.02a | 0.5±0.1b | 0.000 | MRP |
| 127 | 5-Hydroxymethylfurfural | N.D. | 57.0±6.8a | 20.0±0.7b | N.D. | N.D. | N.D. | N.D. | 0.011 | MRP |
| 128 | Safrole | N.D. | N.D. | N.D. | 0.9±0.2a | 0.6±0.1b | 0.5±0.03b | 0.4±0.1b | 0.062 | VFAB |
| 129 | Pyridine | 0.9±0.1c | 1.5±0.3b | 0.8±0.1d | 1.4±0.03b | 0.8±0.3d | 1.1±0.1c | 2.7±0.2a | 0.000 | MRP |
| 130 | 2-Propylpyridine | N.D. | N.D. | 2.9±1.0 | N.D. | N.D. | N.D. | N.D. | N.D. | MRP |
| 131 | 2-Acetylpyrrole | N.D. | N.D. | N.D. | 0.1±0.01c | 0.5±0.2b | 1.3±0.1a | 1.5±0.3a | 0.001 | MRP |
| 132 | Methanethiol | 1.0±0.3e | 47.5±3.7c | 84.1±19.0a | 32.9±2.5d | 51.1±4.1c | 68.5±1.5b | 56.4±2.6b | 0.000 | AADP |
| 133 | Dimethyl disulfide | N.D. | 0.9±0.1b | 5.5±0.5a | N.D. | N.D. | N.D. | N.D. | 0.000 | AADP |
| 134 | 3-Methylthiophene | N.D. | 12.5±1.0c | 7.2±1.3d | 19.5±2.7b | 17.7±1.0b | 13.0±1.5c | 26.3±1.0a | 0.000 | AADP |
| 135 | Dimethyl sulfone | 0.5±0.01b | N.D. | 0.6±0.02a | 0.5±0.1b | 0.3±0.02c | 0.3±0.01c | 0.5±0.1b | 0.001 | AADP |
| 136 | Dimethyl trisulfide | N.D. | 1.4±0.1b | N.D. | N.D. | 1.5±0.2b | 2.1±0.1a | 1.6±0.2b | 0.001 | MRP |
| 137 | 2-Acetylthiazole | N.D. | N.D. | N.D. | 0.9±0.1c | 2.0±0.1a | 0.9±0.3c | 1.4±0.1b | 0.000 | MRP |
| 138 | 2-Thiophenecarboxaldehyde | N.D. | N.D. | 0.2±0.02a | 0.2±0.01a | N.D. | N.D. | N.D. | 0.786 | MRP |
| 139 | Benzothiazole | N.D. | 0.4±0.1a | 0.3±0.01b | N.D. | 0.3±0.02b | 0.3±0.01b | 0.2±0.02b | 0.067 | MRP |
|  | **Total** | 1669.8±97.1c | 8096.5±247.1a | 8546.0±623.7a | 4788.4±201.5b | 4447.1±87.1b | 4414.2±49.5b | 4692.3±58.2b | 0.000 |  |

***Note*:**Results were expressed as mean value ± standard deviation. A row with different letters (a, b, c, d, e, f and g) is significantly different (*P < 0.05*). N.D. meant the volatile compounds were not found in the pork sample. FP, fresh pork; SP1, stewed pork with water; SP2: stewed pork in water and salt; SP3: stewed pork in water, salt and spices; SP4: stewed pork in water, salt, spices and soy sauce; SP5: stewed pork in water, salt, spices, soy sauce and sugar; SP6: stewed pork in water, salt, spices, soy sauce, sugar and cooking wine. Origin1: LOP, Lipid oxidation products; AADP, Amino acid degradation products; MRP, Maillard reaction products; VFAB, Volatiles from aged brine; VFRM, Volatiles from raw meat; UOAC, Unknown origin.

Heterocyclic and sulphur-containing compounds are the important contributions to the formation of flavour in meat products (Shahidi, 1998). As shown in Table 4 - 4, 17 heterocyclic compounds (furan, pyridine and pyrrole) and sulphur-containing compounds were detected in all pork samples. The 2-pentylfuran and safrole were derived from linoleic acid autoxidation (José M Lorenzo, Franco, & Carballo, 2014) and spices (nutmeg, aniseed and ginger), respectively. The 2-pentylfuran is often used as an important indicator of the degree of oxidation of meat product. The contents of 2-pentylfuran in SP1 and SP2 were significantly higher (*P < 0.001*) than that in SP3, SP4, SP5 and SP6, indicating that the stewed pork with only water and salt had a greater effect on lipid oxidation. It has been reported that a large number of furans, pyridines, pyrroles and sulphur-containing compounds could be produced by Maillard reaction and amino acid degradation during cooking (Aaslyng & Meinert, 2017; Zhao et al., 2017). In our study, 3-(4-methyl-3-pentenyl)-furan, furfural, 2-furanmethanol, pyridine, 2-acetylpyrrole and dimethyl trisulfide displayed significantly higher levels (*P < 0.01*) in SP5 and SP6 than those in other groups, which indicated that the addition of sugar and cooking wine could promote the Maillard reaction. For sulphur-containing compounds, methanethiol and dimethyl disulphide from sulphur-containing amino acid degradation were significantly lower in SP1 than that in SP2, which indicated that salt-treated stewed pork was more conducive to the production of sulphur-containing compounds. This result was consistent with that reported by (Liu, Xu, Ouyang, & Zhou, 2006) who found that the levels of sulphur-containing compounds in Nanjing water-boiled salted duck were markedly higher than those in control samples. Regarding 2-acetylthiazole, 2-thiophenecarboxaldehyde and benzothiazole, they originated from Maillard reaction and contributed to roasted, caramel and meaty notes (Tai, & Ho, 1997) for the overall aroma of stewed pork

As shown in Table 4.3, the concentration ratios of 9 classes of volatile compounds in the fresh and stewed pork was presented. Among them, the abundant volatile compounds were aldehydes, alcohols, ketones, hydrocarbons, heterocyclic and sulphur-containing compounds. For stewed pork with different seasonings, the amounts and proportions of aldehydes, heterocyclic and sulphur-containing compounds increased significantly when the pork was stewed in water and salt (SP1 and SP2), while these compounds of pork samples treated with spices, soy sauce, sugar and cooking wine (SP3, SP4, SP5 and SP6) decreased slightly. This result indicates that a large amount of flavour compounds was formed during the thermal processing of pork, however some of them were inhibited due to addition of edible condiments. The number of alcohols and ketones had gradually increased with the addition of seasonings in pork samples, and the content ratios of hydrocarbons in samples SP3, SP4, SP5 and SP6 were much higher than those of samples from other treatment groups. These analyse concluded that heat treatment and seasonings play an important role in cooked pork (Aaslyng & Meinert, 2017; Yang et al., 2018).

The concentration of volatile compounds according to possible origins of the fresh and stewed pork are presented in Figure 4 - 2. It was found that the lipid oxidation, aged brine and amino acid degradation were important origins of volatile compounds in all stewed pork attributed to their contribution to more aroma of the pork samples. For the lipid oxidation and amino acid degradation, their concentrations were highest in SP1 and SP2, followed by SP3, SP4, SP5 and SP6, finally FP. This indicated thatheat-treated pork with water and salt would facilitate lipid oxidation and amino acid degradation to produce more volatiles, while there was an inhibitory effect on heat-treated pork with aged brine, especially spices. Compared with volatile compounds from the aged brine in SP3, SP4, SP5 and SP6, they were significantly higher (*P < 0.05*) than those in SP1 and SP2, this may be due to the addition of food condiments (spices, soy sauce, sugar and cooking wine) in stewed pork.



**Figure 4 - 2:** Concentration of volatile compounds of the fresh and stewed pork. Different letters are significantly different (P < 0.05) in each pork treatment group. FP, fresh pork; SP1, boiled pork in water; SP2, cooked pork in water and salt; SP3, stewed pork in water, salt and spices; SP4, stewed pork in water, salt, spices and soy sauce; SP5, stewed pork in water, salt, spices, soy sauce and sugar; SP6, stewed pork in water, salt, spices, soy sauce, sugar and cooking wine. LOP, Lipid oxidation products; AADP, Amino acid degradation products; MRP, Maillard reaction products; VFAB, Volatiles from aged brine; VFRM, Volatiles from raw meat; UO, Unknown origin.

## *3.3. Odour-active compounds analysis of the fresh and stewed pork*

To evaluate the contributions of volatile compounds to overall flavour of the fresh and stewed pork, the OAVs of these compounds were determined by dividing the concentration of the compound by its odour threshold in water. As can be seen from Table 4 - 5, a total of 29 odour-active compounds with OAVs greater than 1 were selected from 139 volatile compounds, including 14 aldehydes, 4 alcohols, 3 ketones, 1 hydrocarbon, 1 ester, 2 ethers, 1 phenol, 3 furans, N- or S-containing compounds. 7 of them with relatively high OAVs were detected in all samples: hexanal (OAV at 213.9-524.2), nonanal (OAV at 248.7-454.6), 1-octen-3-ol (OAV at 56.9-194.3), dimethyl disulfide (OAV at 76.8-141.3), heptanal (OAV at 19.0-41.7), 2-pentylfuran (OAV at 18.1-40.7) and 2-ethylfuran (OAV at 17.3-22.9). These compounds were known as the key odour-active compounds due to their significant contributions to the integral flavour. Furthermore, it was found that the OAVs of hexanal, heptanal, octanal, nonanal and decanal increased significantly (*P < 0.01*) in SP1 and SP2.

Statistical analysis showed that the total OAVs of odour-active compounds of SP1 and SP2 were significantly higher (*P < 0.001*) than those of FP and SP3, SP4, SP5 and SP6. Linear aldehydes like pentanal, hexanal, hepanal, octanal, nonanal and decanal have been reported to be generated from lipid oxidation (Petričević et al., 2018). Moreover, these aldehyde compounds could be detected in different processing methods and may contribute grassy, fatty and fruity notes to overall aroma of the pork samples. Unsaturated aldehydes such as (Z)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E,E*)-2,4-nonadienal, 2-undecenal and (*E,E*)-2,4-decadienal are degradation products of linoleate and linolenate hydroperoxides (Zhou, Chong, Ding, Gu, & Liu, 2016). Among them, only 2-undecenal has not significantly differences (*P > 0.05*) in FP, SP1 and SP2 indicating that there was no influence on the formation of 2-undecenal during heating and salt treatment. On the other hand, the rest of the olefin aldehydes have relatively higher OAVs in SP1 and SP2. This showed that SP1 and SP2 could promote the increase of some unsaturated aldehydes. Benzeneacetaldehyde, with honey and sweet notes, is a well-known aroma component formed from Maillard reaction of phenylalanine (Lotfy, Fadel, El-Ghorab, & Shaheen, 2015) and the OAV in SP5 were significantly higher (*P < 0.001*) than that in other samples. 1,8-Cineole, anethole and estragole, with mint and aniseed flavour, were the most abundant in SP3. The OAVs of 1-octen-3-ol, (*E*)-2-octen-1-ol, 2,3-butanedione, 2,3-octanedione and 2-pentylfuran were the highest in SP1 and SP2, followed by SP3, SP4, SP5 and SP6, of which 1-octen-3-ol and 2,3-octanedione was shown to be richer in SP2 than that in SP1. Linalool and eugenol were found immediately when the spices were added to the cooked pork, which may be due to the flavour of the star anise itself. Dimethyl trisulfide, with fish and cabbage notes, was considered as the main sulphur-compound in SP1, SP4, SP5 and SP6.

**Table 4 - 5:** Odour-active compounds (OAVs > 1) in the fresh and stewed pork.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Compounds | 1Odour description;  2odour threshold (μg·kg-1) | FP | SP1 | SP2 | SP3 | SP4 | SP5 | SP6 | *p* value |
| 4 | Pentanal | Almond, pungent; 9 | 3.1±0.4e | 6.0±0.8d | 8.3±0.1ab | 9.0±0.4a | 6.7±1.1cd | 7.8±0.6bc | 7.3±0.4bc | 0.000 |
| 5 | Hexanal | Grass, fat; 4 | 0.9±0.3e | 158.3±3.3a | 158.5±3.7a | 58.3±2.2c | 63.5±2.5b | 44.1±0.2d | 55.3±3.9c | 0.000 |
| 6 | Heptanal | Fat, citrus; 3 | 18.9±1.1c | 40.9±5.3b | 53.4±0.7a | 10.5±0.6d | 12.3±0.4d | 12.8±1.2d | 12.7±2.4d | 0.000 |
| 7 | Octanal | Fat, lemon, green; 0.7 | 121.0±15.5c | 260.0±14.8b | 329.9±35.8a | 89.2±7.1d | 123.4±13.8c | 72.2±6.1d | 78.5±12.3d | 0.000 |
| 8 | (Z)-2-Heptenal | Fishy; 13.5 | N.D. | 0.4±0.01b | 2.9±0.7a | N.D. | N.D. | N.D. | N.D. | 0.024 |
| 10 | Nonanal | Fat, citrus, green; 1 | 219.8±13.3cd | 844.4±94.8b | 1475.5±124.6a | 316.3±5.8c | 171.4±17.8d | 110.3±7.9d | 284.2±11.9c | 0.000 |
| 11 | (*E*)-2-Octenal | Green, nut, fat; 3 | 8.2±0.4c | 25.3±0.4a | 14.1±2.5b | 1.1±0.1d | 1.8±0.4d | 2.2±0.6d | 2.8±0.2d | 0.000 |
| 12 | Decanal | Soap, orange peel; 2 | 2.6±0.3bc | 4.5±0.9a | 4.0±0.3a | 2.2±0.1bc | 2.3±0.3bc | 2.1±0.1c | 3.0±0.2b | 0.001 |
| 14 | (*E*)-2-Nonenal | Cucumber, green; 0.19 | 39.2±5.0c | 59.5±13.3b | 79.5±5.2a | 8.6±0.6d | 9.7±3.6d | 12.6±1.9d | 10.0±2.4d | 0.000 |
| 16 | Benzeneacetaldehyde | Honey, sweet; 4 | 0.6±0.03d | 1.0±0.2c | 1.5±0.4b | 0.9±0.1cd | 1.8±0.1b | 2.4±0.3a | 1.6±0.1b | 0.000 |
| 17 | (*E*)-2-Decenal | Orange; 0.3 | 9.8±0.7c | 18.6±3.6a | 15.4±3.4b | 3.3±0.5d | 2.0±0.1d | 2.5±0.4d | 2.7±0.2d | 0.000 |
| 19 | (*E,E*)-2,4-Nonadienal | Geranium, pungent; 0.09 | 30.1±3.0b | 67.7±3.2a | 64.0±2.5a | N.D. | N.D. | N.D. | N.D. | 0.000 |
| 20 | 2-Undecenal | Sweet; 0.78 | 3.3±0.3a | 3.5±0.9a | 3.1±0.4a | N.D. | N.D. | N.D. | N.D. | 0.717 |
| 22 | (*E,E*)-2,4-Decadienal | Fried, wax, fat; 0.07 | 104.3±14.7b | 143.3±7.6a | 113.4±13.1b | 10.7±0.6c | N.D. | 12.4±2.9c | 22.7±1.9c | 0.000 |
| 34 | 1,8-Cineole | Mint, sweet; 1 | 1.7±0.8e | 9.6±0.8d | 15.2±1.8d | 123.2±11.5a | 63.6±1.4b | 66.5±0.4b | 43.0±1.1c | 0.000 |
| 38 | 1-Octen-3-ol | Mushroom; 2 | 0.7±0.2g | 65.0±2.2b | 68.5±1.5a | 25.3±0.3d | 29.7±0.2c | 13.9±1.0f | 20.1±1.8e | 0.000 |
| 41 | Linalool | Flower, lavender; 6 | N.D. | N.D. | N.D. | 12.6±1.0a | 3.8±0.3b | 3.7±0.2b | 3.6±0.4b | 0.000 |
| 45 | (*E*)-2-Octen-1-ol | Soap, plastic; 50 | 0.8±0.1c | 1.5±0.2a | 1.2±0.2b | 0.3±0.03d | 0.3±0.03d | 0.3±0.04d | 0.3±0.01d | 0.000 |
| 55 | 2,3-Butanedione | Butter; 4.37 | 2.9±0.5bc | 5.4±0.4a | 5.3±0.7a | 5.6±0.5a | N.D. | 3.4±0.3b | 2.5±0.1c | 0.000 |
| 60 | 1-Hydroxy-2-propanone | Sweet, pungent; 10 | N.D. | 2.1±0.4d | 5.8±1.5b | 3.0±0.2d | 5.5±0.7bc | 7.3±0.2a | 4.3±0.2c | 0.000 |
| 61 | 2,3-Octanedione | Green, woody; 12 | 25.3±1.7c | 48.2±2.2b | 93.5±15.4a | 26.8±0.8c | 28.3±0.6c | N.D. | 24.8±1.3c | 0.000 |
| 80 | D-Limonene | Citrus, mint; 10 | N.D. | 1.3±0.2c | 2.2±0.1b | 1.3±0.2c | 2.2±0.2b | 3.0±0.3a | 1.3±0.1c | 0.000 |
| 98 | Ethyl acetate | Pineapple; 5 | N.D. | 9.3±0.8c | 14.0±0.8a | 7.1±1.5d | 11.8±0.2b | 6.9±0.8d | 9.6±0.2c | 0.000 |
| 111 | Anethole | Anissed-like; 15 | 0.2±0.03e | 1.1±0.04d | 1.1±0.1d | 5.4±0.1a | 2.2±0.2b | 2.3±0.1b | 1.5±0.1c | 0.000 |
| 112 | Estragole | Licorice, anise; 6 | N.D. | N.D. | 0.1±0.02c | 5.2±0.3a | 0.3±0.03bc | 0.5±0.1b | 0.4±0.1b | 0.000 |
| 117 | Eugenol | Clove, honey; 7.1 | N.D. | N.D. | N.D. | 3.0±0.4b | 2.6±0.1b | 4.1±0.1a | 1.3±0.1c | 0.000 |
| 123 | 2-Pentylfuran | Green bean, butter; 6 | N.D. | 178.6±18.1a | 162.6±29.7a | 41.5±4.7b | 41.7±3.6b | 39.1±0.5b | 39.0±2.4b | 0.001 |
| 132 | Methanethiol | Sulfur, gasoline, garlic; 1.05 | 0.9±0.3e | 45.2±3.5c | 80.1±18.1a | 31.3±2.4d | 48.7±3.9c | 65.3±1.5b | 53.7±2.5bc | 0.000 |
| 136 | Dimethyl trisulfide | Sulfur, fish, cabbage; 0.01 | N.D. | 136.7±9.1b | N.D. | N.D. | 151.3±15.9b | 212.4±12.5a | 163.4±17.2b | 0.001 |
|  | **Total** |  | 594.6±58.6d | 2136.6±186.9b | 2773.0±263.3a | 801.7±42.0c | 786.9±67.4c | 710.1±43.1cd | 849.6±63.5c | 0.000 |

***Note:*** Each value is expressed as mean *±* SD; N.D. = not detected. a–e Different letters in the same row indicate that there is significant difference (*P <* 0.05, along the lines). FP, fresh pork; SP1, stewed pork with water; SP2: stewed pork in water and salt; SP3: stewed pork in water, salt and spices; SP4: stewed pork in water, salt, spices and soy sauce; SP5: stewed pork in water, salt, spices, soy sauce and sugar; SP6: stewed pork in water, salt, spices, soy sauce, sugar and cooking wine.

1 Odor thresholds were mainly obtained from online database, (http://www.flavornet.org, <http://www.odour.org.uk>).

2 Odor descriptions were mainly gathered from online database, (http://www.flavornet.org).

## *3.4. PCA and PLS-DA analysis of odour-active compounds*

In order to clarify the differences in aroma profile of the fresh and stewed pork, a principal component analysis (PCA) was performed and showed in Figure 4 - 3a-b. The first principal component (PC1) explained 55.17%, the second principal component (PC2) explained 22.85% and the third principal component (PC3) explained 13.22% of the variations. The first three principal components (PCs) accounted for 91.24% of the total variance and were sufficient to explain the maximum variation in all original data of the pork samples. As can be seen in Figure 4 - 3a, the PC1 and PC2 showed a clear-cut separation of the samples into three major groups. Among them, sample dot of FP was located in the fourth quadrant, and sample dots representing SP3, SP4, SP5 and SP6 were located in the second quadrant and could be recognized as one cluster, while sample dots of SP1 and SP2 located in the first and fourth quadrant were considered as a group because of their relatively closer distance. As shown in Figure 4 - 3b, the fresh and stewed pork samples were divided into four groups in PC1 and PC3, that is, group I: FP; group II: SP1 and SP2; group III: SP3; group IV: SP4, SP5 and SP6. The four group of sample points were located in the different quadrants indicating that the overall aroma of each group of samples were different. Besides, SP4, SP5 and SP6 samples was close and located in the third quadrant. It can be concluded that the overall flavour of SP4, SP5 and SP6 was similar each other. Similarly, so was SP1 and SP2 samples.

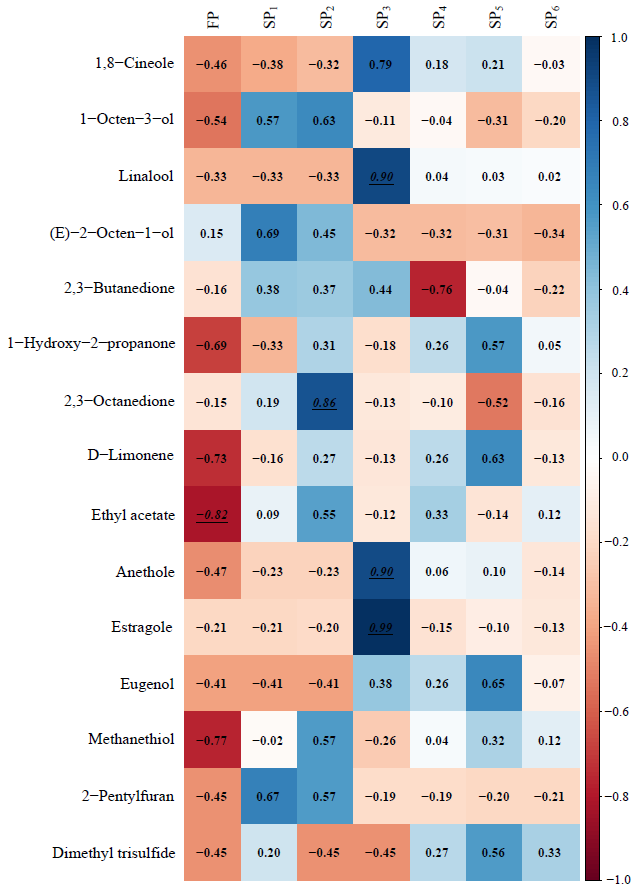
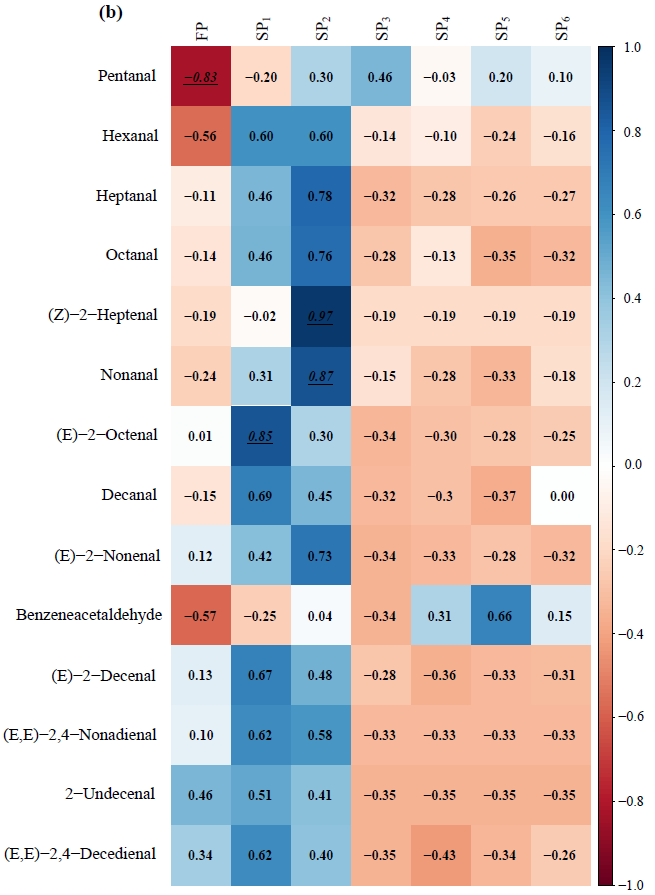
The supervised PLS-DA of volatile compounds of the stewed pork gave some interesting information about differences between them. As shown in Figure 4 - 4a, except for SP3, SP4, SP5 and SP6, only the separation was observed for FP, SP1 and SP2 (R2X = 0.968, R2Y = 0.818 and Q2 = 0.628). SP3, SP4, SP5 and SP6 were located on the negative side of axis 1, whereas FP, SP1 and SP2 were founds the positive side of axis1, SP1 and SP2 were close each other. Obviously, the different stewed pork was separated into three group (SP3, SP4, SP5 and SP6, SP1 and SP2, FP). It could also be concluded that the overall flavour of SP3, SP4, SP5 and SP6, SP1 and SP2, FP were greatly different, and each group samples possessed the similar flavour profiles. The result is consistent with the PCA analysis (Figure 4 - 3a). In addition, to identify the most discriminative volatiles contributing to the observed the fresh and stewed pork samples, variable identification (VID) coefficients were calculated (Figure 4 - 3b). Volatiles with VID ≥ |0.80| discriminating FP, SP1, SP2 and SP3 were predominantly aldehyde compounds (pentanal, (Z)-2-heptenal, nonanal and (E)-2-octenal) and related volatiles (linalool, 2,3-octanedione, ethyl acetate, anethole and estragole). Moreover, no compounds with VID ≥ |0.80| were found discriminating SP4, SP5 and SP6. To further study the relationship between the odour-active compounds and the pork samples, the heat map of correlation coefficients was presented in Figure 4 - 4b. As shown in Fig. 4.4a-b, It can be observed that FP was not only on the opposite side of pentanal, 1-hydroxy-2-propanone, D-limonene, ethyl acetate and methanethiol, but also strongly and negatively correlated with them (-0.83 ≤ r ≤ -0.69), while the left 24 odour-active compounds (-0.57 ≤ r ≤ 0.46) were low relevant to samples FP. Most of odour-active compounds were close to SP1-2 on the right side of t1 and the strong and positive correlation were showed between SP1 and SP2, hexanal, heptanal, octanal, (Z)-2-heptenal, nonanal, (*E*)-2-octenal, decanal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,4-decedienal, 1-octen-3-ol, (*E*)-2-octen-1-ol, 2,3-octanedione and 2-pentylfuran (0.60 ≤ r ≤ 0.97). Moreover, SP2 induced an increase in the correlation coefficients of heptanal, octanal, (Z)-2-heptenal, (*E*)-2-nonenal, 1-octen-3-ol and 2,3-octanedione indicating that the addition of salt during the processing of stewed pork was beneficial to the formation of these compounds. For SP3, SP4, SP5 and SP6, benzeneacetaldehyde, 1,8-cineole, linalool, D-limonene, anethole, estragole and eugenol had the high and positive correlation coefficients (0.63 ≤ r ≤ 0.99) on the left side of t1, only 2,3-butanedione had the high and negative correlation coefficient (r = -0.76). Because 1,8-cineole, linalool, anethole and estragole had a higher correlation coefficient (0.79 ≤ r ≤ 0.99) with SP3 and a lower correlation coefficient (-0.15 ≤ r ≤ 0.21) with SP4, SP5 and SP6, this suggests that they may be potential flavour markers to distinguish SP3 and SP4, SP5 and SP6.

**(a)**

**(b)**

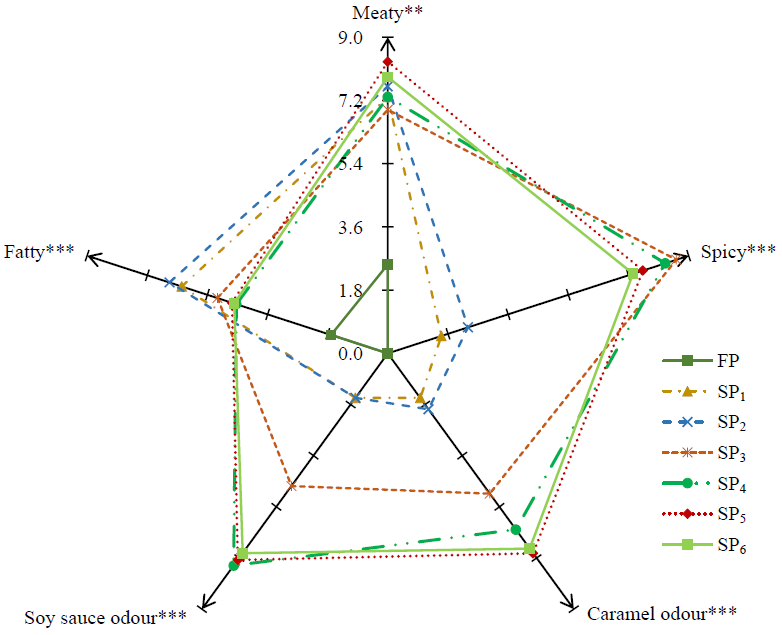
**Figure 4 - 3:** Score plots of PCA of the fresh and stewed pork. (a) PC1 plotted against PC2 and (b) PC1 against PC3. FP, fresh pork; SP1, stewed pork with water; SP2: stewed pork in water and salt; SP3: stewed pork in water, salt and spices; SP4: stewed pork in water, salt, spices and soy sauce; SP5: stewed pork in water, salt, spices, soy sauce and sugar; SP6: stewed pork in water, salt, spices, soy sauce, sugar and cooking wine.

**(a)**



**Figure 4 - 4:** **(a)** Loading biplot of t1 and t2 of the model performed after PLS-DA of the volatile compounds in different pork samples. **(b)** Heat map of the correlations between volatile compounds and the pork samples.

## *3.5. Descriptive sensory analysis*

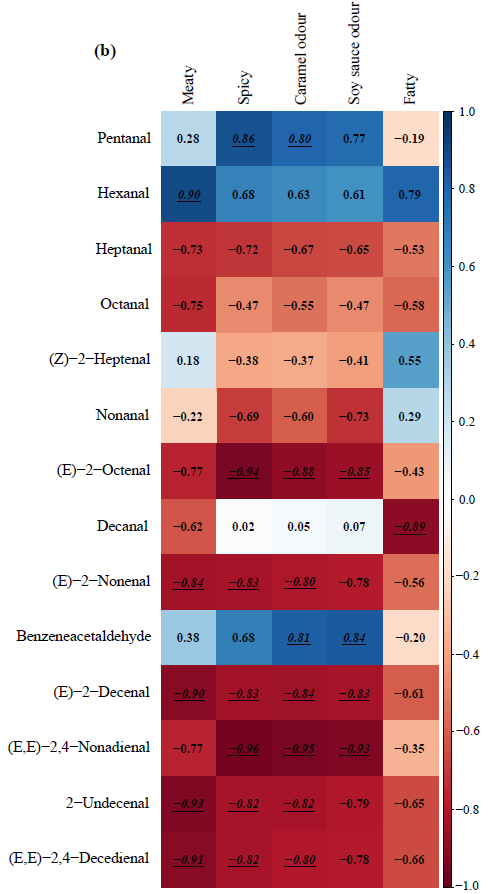
To describe the differences of odour profiles of seven kinds of pork samples, the flavour sensory evaluation was performed using the five representative descriptors, namely, “meaty”, “spicy”, “caramel”, “soy sauce” and “fatty”. ANOVA was used to distinguish differences between the fresh and stewed pork through descriptive sensory analysis. As can be seen from Figure 4 - 5. Significant differences (*P ≤ 0.01*) were found in five odour attributes of the pork samples. The intensities of fatty notes in SP1 and SP2 were highest, followed by SP3, SP4, SP5, SP6 and FP. As described by the panellists from GC-MS/O, hexanal, heptanal, octanal, (*Z*)-2-heptenal, nonanal, (*E*)-2-octenal, decanal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,4-decedienal and 2-pentylfuran might be closely related to the fatty odour. This result was in agreement with Figure 4 – 4b. The meaty and caramel odour of SP5 and SP6 had the highest score in all samples, which could be mainly attributed to furans and N-containing compounds such as 3-(4-methyl-3-pentenyl)-furan, furfural, 2-furanmethanol, pyridine and 2-acetylpryrrole. In additional, the strong spicy ****and soy sauce smell was presented in SP3 and SP5, respectively.

**Figure 4 - 5:** The odour sensory profiles of the fresh and stewed pork.

## *3.6. Relationship between sensory evaluation and odour-active compounds*

To the best of our knowledge, there was the potential correlation between flavour intensity and odour-active compounds. Therefore, PLSR was employed to establish relationship between the five sensory descriptors of the fresh and stewed pork and the odour-active compounds analysed by GC-MS/O and GC × GC-TOFMS, and the correlation coefficient between them was expressed in the heat map. As shown in Figure 4 - 6a, most of the *X*-matrix (contribution ratios of the odour-active compounds) and *Y*-matrix (intensities of the sensory attributes) are loaded around the circle (*r*2 = 100%, *r*2 = represent the degree of interpretation). The model quality (Q2 = 0.846) ≥ 0.50 indicated that they were well explained by the PLSR model. The first two PLSR components explained 74.0% of *X*-matrix and 92.7% of *Y*-matrix. The dots corresponding to sample SP1 and SP2 had overlap in the second quadrant, and the samples points of SP3, SP4, SP5 and SP6 were close in the fourth quadrant, and as well as FP was found in the third quadrant. So, the fresh and stewed pork samples can be divided into three group and this result was consistent with previous PCA plots (Figure 4 - 3a). According to Figure 4 - 6a-b, it can be observed that SP3, SP4, SP5 and SP6 were characterized by soy sauce, caramel and spicy odour because of their short distance, and the three aroma attributes aforementioned had positively correlated with pentanal, benzeneacetaldehyde, 1,8-cineole, 1-hydroxy-2-propanone, D-limonene, ethyl acetate, eugenol, methanethiol and dimethyl trisulfide with a high correlation coefficient (0.60 ≤ r ≤0.92). In contrast, soy sauce, caramel and spicy notes, located on the bottom right side of the loading plot, were strongly and negatively correlated with some aldehydes (heptanal, nonanal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E,E*)-2,4-nonadienal, 2-undecenal and (*E,E*)-2,4-nonadienal) and unsaturated alcohols like (*E*)-2-ocetn-1-ol. SP1 and SP2 on the top left sided of loading plot was mainly descripted fatty and meaty odour, which was in accordance with the descriptive sensory analysis. The two attributes were highly associated with hexanal, 1-octen-3-ol and 2-pentylfuruan. Besides, FP was located far from these flavour attributions and most volatiles, which indicated that there was not the unique flavour of the fresh pork.

**(a)**



**Figure 4 - 6: (a)** PLSR loading for the odour attributes and the odour-active compounds of the fresh and stewed pork. **(b)** Heat map illustrating the Pearson correlation between descriptor intensities and proportion.

# 4. Conclusions

The volatile profile of fresh and stewed pork could be more fully characterized using GC-MS/O and GC × GC-TOFMS analysis. A total of 139 volatile compounds were identified from all stewed pork and 7 of which were confirmed as the key odour-active compounds, namely, hexanal, nonanal, 1-octen-3-ol, dimethyl disulphide, heptanal and 2-pentylfuran and 2-ethylfuran. The fresh and stewed pork could be classified to three groups (FP; SP1 and SP2; SP3, SP4, SP5 and SP6) by PCA and PLS-DA analysis based on the odour-active compounds. This result showed that the volatile profile of pork stewed in water and salt possessed the similar flavour, and the flavour composition of stewed pork with spices, soy sauce, sugar and cooking wine was not significantly different, however there were significant differences in the overall flavour between pork samples of different groups. In addition, the most discriminative volatiles of stewed pork could be well confirmed by PLS-DA. The relationship between odour-active compounds and sensory evaluation were evaluated by PLSR, which indicated that pentanal, benzeneacetaldehyde, 1,8-cineole, 1-hydroxy-2-propanone, D-limonene, ethyl acetate, eugenol, methanethiol and dimethyl trisulfide were positively correlated with soy sauce, caramel and spicy notes. On the contrary, soy sauce, caramel and spicy odours were strongly and negatively correlated with heptanal, nonanal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E,E*)-2,4-nonadienal, 2-undecenal, (*E,E*)-2,4-nonadienal and (*E*)-2-ocetn-1-ol.

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**5**

# Chapter Ⅴ. Study on the flavour compounds of stewed pork with different processing methods

*The processing methods of stewed pork also affect the formation of pork flavor. During the processing of stewed pork, the flavor compounds are often lost and volatilized. In order to solve this problem, we discussed the influence of different processing methods on the flavor of stewed pork. The aim of this chapter is to find a better method to improve special flavor of stewed pork.*

This article will be submitted for publication in Journal of the Science of Food and Agriculture.

**Abstract:** The objective of this study was to investigate the volatile and non-volatile profile of stewed pork with different processing methods (TS: traditional stewing, TSE: traditional stewing with enzymatic degradation, TSE: traditional stewing with enzymatic degradation and Maillard reaction, HS: high-temperature stewing, HSE: high-temperature stewing with enzymatic degradation, HSEM: high-temperature stewing with enzymatic degradation and Maillard reaction). The volatile compounds, electronic nose, free amino acids (FAAs), 5’-nucleotides, fatty acids (FAs) and electronic tongue of stewed pork were determined. Results showed that the high-temperature stewed pork (HS, HSE and HSEM) had a higher content of volatile composition than traditional stewed pork (TS, TSE and TSEM), especially sample HSEM. All stewed pork from traditional and high-temperature stewing method were classified into two group using electronic nose due to different flavour characteristic. The contents of umami amino acids (UAAs), sweet amino acids (SAAs) and bitter amino acids (BAAs) of high-temperature stewed pork were higher significantly (*P < 0.05*) than those of traditional stewed pork, of which the content of Asp and Glu related to umami taste were the most in sample HS and HSEM. The high-temperature stewed pork showed the lower contents of 5’-nucleotides and FAs than traditional stewed pork. It may be because high-temperature heat treatment induced fat oxidation and nucleotide degradation to produce more flavour compounds. These founding indicated that the pork with high-temperature stewing (HS, HSE and HSEM) could be used as an effective method to improve taste and flavour of stewed pork, sample HSEM was outstanding in the formation of odour compounds.

**Keywords:** traditional stewed pork, high-temperature stewed pork, enzymatic degradation, Maillard reaction, flavour compounds

# 1. Introduction

The stewed pork is a traditional sauce pickled product in China and processed by boiling hind leg meat with various condiments and spices for a long time. The stewed pork products are popular with consumers owing to the unique aroma and taste profile. Flavour is the most important factor for sensory attributes with regard to eating quality of stewed meat products (Qi, Liu, Zhou, & Xu, 2017) and closely related to volatile compounds and non-volatile compounds (Dashdorj, Amna, & Hwang, 2015; Kosowska, A. Majcher, & Fortuna, 2017). The volatile compounds include aldehydes, alcohols, ketones, hydrocarbons, esters, ethers, furans, N- and S- containing compounds generated from many chemical reactions (e. g. lipid oxidation, Maillard reaction and lipid-Maillard interactions) (Aaslyng & Meinert, 2017; Han, Zhang, Fauconnier, & Mi, 2019). The non-volatile compound precursors were mainly reducing sugar, free amino acids (FAAs), nucleotides and fatty acids (FAs) (Maughan & Martini, 2012). As reported by Li et al., (2016), the flavour compounds were continuously volatilized and outflowed during the processing of stewed meat to reduce the flavour quality of meat products. Although our research team had proposed the quantitative stewing method to keep its characteristic aroma, in order to obtain the more satisfactory flavour, it is also necessary to find a better way to improve special flavour.

To our knowledge, lots of flavour precursors formed by enzymatic degradation were involved Maillard reaction. Several studies have reported that the meat flavour was prepared by using FlavourzymeTM, Trypsase and protamexTM to hydrolyse chicken and beef bones, thereby improving the aroma of the meat (Han et al., 2014; Xu, You, Song, Gong, & Pan, 2018). The Maillard reaction typically occurs between amino acids and reducing sugars, and eventually results a large number of volatile compounds responsible for the special aroma in meat products (Jayasena, Ahn, Nam, & Jo, 2013). D-xylose, L-cysteine and thiamine are important precursors to generate meat-flavoured sulphur-containing odorants (Aaslyng & Meinert, 2017) in Maillard reaction system. The research has showed that several main flavour compounds were identified in the model systems of D-xylose, L-cysteine and thiamin. It has been reported that 3-Mercapto-2-pentanone (MP), 2-methyl-3-furanthiol (MFT), 4,5-dihydro-2-methyl-3-furanthiol and 4-methyl-5-thiazoleethanol have been identified in these model reaction, which presented intense roasted, coffee-like and meat-like notes (Hofmann & Schieberle, 1995). These results indicated that the enzymatic hydrolysis and Maillard model reaction were usually used to generate dominated aroma compounds. Therefore, this is the feasible way to enhance the flavour of stewed pork by enzymatic hydrolysis and Maillard model reaction. In our study, the pork was stewed at higher temperature, which could facilitate the Maillard reaction process.

The aim of this study was to analyse the differences of volatile compounds and taste compounds (FAAs, nucleotides, FAs) in the pork from traditional and high-temperature stewing combined with enzymatic degradation and Maillard reaction. Additionally, it was expected to provide an effective way to improve the aroma of stewed meat products.

# 2. Materials and methods

## *2.1. Materials and chemicals*

The hind leg muscle of pigs was obtained from Chuying Agro-Pastoral Group Co. Ltd. (Zhengzhou, Henan Province, China) and stored at -20°C until use. Pigs belonged to Duroc × (Landrace × Yorkshire) pig breed (DLY, n = 18, aged 5-6 months and with body weights of 90-95 kg). All pigs were fed under the same rearing conditions and slaughtered following routine abattoir procedures. The salt and mixed spices were obtained from the local market (Beijing, China). Flavourzyme TM (25000 U/g) was purchased from Novozymes Co. Ltd (Beijing, China). The standard of free amino acids, 5’-nucleotide and 2-methyl-3-heptanone were purchased form Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). D-xylose, L-cysteine and thiamine were analytical grade and bought from Sinopharm Chemical Reagent Beijing Co. Ltd (Beijing, China).

## *2.2. Stewed pork with different processing methods*

### 2.2.1. Traditional stewing with enzymatic degradation and Maillard reaction (TSEM)

The frozen pork was thawed overnight at 4 °C and the skin, visible fat and connective tissues were removed. About 1 kg of pork were cut into small pieces (5.0 cm × 4.0 cm × 3.0 cm) and were stewed at 100 °C by adding 2 L water, 30 g salt and 12 g mixed spices. Subsequently, the mixed liquid was obtained through filtering the spices. The flavourzyme was added to mixed liquid at a ratio of 0.075‰ (w/w), then 10% flavourzyme mixture (w/w) were injected to the pork, and finally stirred evenly in the tumble machine at room temperature for 60 min. The tumbled pork was stewed for 45 min at 98 ± 2 °C in the brine and soaked for 60 min. This brine contained salt (60 g/kg pork), mixed spices (12 g/kg pork), 5‰ D-xylose (w/w, based on pork weight), 1‰ L-cysteine (w/w, based on pork weight) and 3‰ thiamine (w/w, based on pork weight). The brine of traditional stewing with enzymatic degradation (TSE) included salt (60 g/kg pork) and mixed spices (12 g/kg pork). The pork processing technology was consistent with TSEM. Compared with TSEM, 10% water (w/w) were injected to the pork and the brine of traditional stewing (TS) only contained salt (60 g/kg pork) and mixed spices (12 g/kg pork). The other processes are the same. The processing flow chart of traditional stewed pork is shown in Figure 5 - 1a-b.

### 2.2.2. High-temperature stewing with enzymatic degradation and Maillard reaction (HSEM)

The frozen pork was thawed overnight at 4 °C and then cut into small pieces (5.0 cm × 4.0 cm × 3.0 cm). The pork was tumbled at room temperature for 60 min after injected with 10% the brine (w/w). The brine contained salt (60 g/kg pork), mixed spices (12 g/kg pork), 0.15‰ flavourzyme (w/w, based on pork weight), 5‰ D-xylose (w/w, based on pork weight), 1‰ L-cysteine (w/w, based on pork weight) and 3‰ thiamine (w/w, based on pork weight). The roasting process was 30 min at 90 °C to dry the surface to avoid water exudation from internal tissue. The steaming process was 5 min at 120 °C. Finally, the stewed pork was roasted at 90 °C for 25 min to dry the surface moisture. The brine of high-temperature stewing with enzymatic degradation (HSE) was contained salt (60 g/kg pork), mixed spices (12 g/kg pork) and 0.15‰ flavourzyme (w/w, based on pork weight). The other operations were the same as HSEM. Compared to HSEM, the brine of traditional stewing (HS) only contained salt (60 g/kg pork) and mixed spices (12 g/kg pork), and other processes are the same. The processing flow chart of high-temperature stewed pork is shown in Figure 5 - 1c.

(a)

Cooked for 45 min at 98 ± 2 °C

Soaked for 60 min

Stirred for 60 min in tumble machine

Added flavourzyme

Water, salt and mixed spices

Fresh pork

100 °C for 10 min

Boiled pork

Cooked liquid

Reaction substrate

Injected into pork

Pork samples

Stewed pork

Different brine: (1) 2 L/kg water, 60 g/kg salt, 12 g/kg mixed spices, 5‰ D-xylose, 1‰ L-cysteine and 3‰ thiamine (TSEM); (2) 2 L/kg water, 60 g/kg salt and 12 g/kg mixed spices (TSE). (Unit: w/w, based on pork weight).

(b)

Soaked for 60 min

Cooked for 45 min at 98 ± 2 °C

No added flavourzyme

Stirred for 60 min in tumble machine

100 °C for 10 min

Water, salt and mixed spices

Fresh pork

Boiled pork

Cooked liquid

Injected into pork

Pork samples

Stewed pork

(3) 2 L/kg water, 60 g/kg salt, 12 g/kg mixed spices (TS). (Unit: w/w, based on pork weight).

(c)

Injected different brine

Fresh pork

Stirred for 60 min in tumble machine

(1) 2 L/kg water, 60 g/kg salt, 12 g/kg mixed spices, 0.15‰ flavourzyme, 5‰ D-xylose, 1‰ L-cysteine and 3‰ thiamine (HSEM); (2) 2 L/kg water, 60 g/kg salt, 12 g/kg mixed spices, 0.15‰ flavourzyme (HSE); (3) 2 L/kg water, 60 g/kg salt, 12 g/kg mixed spices (HS). (Unit: w/w, based on pork weight).

Roasted for 30 min at 90 °C

Steamed for 5 min at 120 °C

Roasted for 25 min at 90 °C

Stewed pork

**Figure 5 - 1:** The processing flow chart of stewed pork. a-b: traditional stewing, c: high-temperature stewing.

## *2.3. Volatile compounds of different stewed pork*

### 2.3.1. Volatile compounds analysis by GC/MS-O

The solid-phase micro-extraction (SPME) methods was used to extract the volatile compounds from the stewed pork samples. 5.0 g of the sample was placed into a 40 mL headspace via, and 1μL of 2-methyl-3-heptanone solution with a concentration of 0.816 μg/μL was added as an internal standard. This via was equilibrated in a thermostatic water bath at 60 °C for 20 min. A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, Inc., Bellefonte, PA, USA) was inserted and headspace absorption was performed for 40 min. Upon completion, the fiber was inserted into the injection port (250 °C) of the GC instrument to desorb the analyses for 5 min. All samples were extracted in triplicate.

The volatile compounds of stewed pork were analysed and identified by a gas chromatography-mass spectrometry (GC-MS) instrument (7890A-7000B, Agilent Technologise, Inc., Santa Clara, CA, USA) equipped with an olfactory detection port (Sniffer 9000; Brechbuhler, Schlieren, Switzerland). Capillary column DB-wax (30 m × 0.32 mm i.d., 0.25 µm film thickness; J & W Scientific, Inc., Folsom, CA, USA) was used with helium (purity of ≥99.999%) as the carrier gas at 1.2 mL/min flow rate. The front inlet temperature was 250 °C with a solvent delay of 4 min. The heating program was as follows: the initial temperature was 40 °C for 3 min, ramped to 200 °C at a rate of 5°C/min, then ramped to 240 °C at a rate of 10 °C/min with a 3 min final hold. The infector mode was splitless. The transfer line temperature and ion source temperature were kept a 240 °C and 230 °C. Electro-impact mass spectra were generated at 70 eV, with *m/z* scan range from 50 to 400 amu. A sniffing port (Sniffer 9000) coupled to a GC-MS instrument was used for odour-active compound characterization. The effluent from the capillary column was split 1:1 (*v/v*) between the mass spectrometry detector and the olfactory detector port. A panel that contains eight trained staff was utilized for the sniffing test on GC-O.

Volatile compounds were identified based on a comparison of GC retention indices (RI) with authentic compounds, mass spectra (comparison with MS spectra database NIST 2.0 mass spectra libraries installed in the GC-MS equipment) and odour descriptions in the literature and online databases (http://www.favornet.org; http://www.odour.org.uk). Quantitative data of the identified compounds were obtained by the calibration curves of GC-peak area and the amount ratios for the target analyte relative to 2-methyl-3-heptanone according to the method of (Han et al., 2019).

### 2.3.2. Electronic nose analysis

The odour profile of stewed pork was analysed by a portable electronic nose (PEN 3, Win Muster Airsense Analytics, Inc., Schwerin, Germany), which included ten types of metal oxide semiconductors for specific recognition of different volatile compound classes. The performance description and sensitivity of all sensors was present Table 5 - 1. 1.0 g of stewed pork sample was into 10 mL airtight vials and sealed for testing. A filtered and dried air flow (99%, 300 ml/min) was used as a carrier gas for E-nose detection. The measurement time was 60 s, and the standby time was 180 s.

**Table 5 - 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 |

## *2.4. Taste compounds of different stewed pork*

### 2.4.1. Determination of free amino acids

FAAs were extracted in accordance with the method of our precious study (Li et al., 2016). Briefly, 5.0 g of stewed pork samples were dissolved in 20 mL of ultra-pure water and homogenized at 0 °C for 1 min in an Ultra Turrax T10 (IKA, Königswinter Germany). Subsequently, 20 mL of 5% cold trichloroacetic acid was added to the homogenate, and the mixture was stored at 4°C for 12 h. The supernatants were filtered through a 0.45-µm membrane prior further analysis. Derivation of amino acids was carried out following the procedure described in the “AcQQ-Tag” kit. Firstly, 20 µL of the extract or of the amino acid standards were transferred to a 1.5 mL amber glass vial with a Teflon-lined septum and mixed with 60 µL of AccQ.Fluor borate buffer. Then 20 µL of reconstituted AccQ.Fluor reagent was added and the mixtures were then heated at 55 °C for 10 min. FAA analysis was conducted using a high performance liquid chromatograph (HPLC) (Agilent 1200 series, Agilent Technologies, Palo Alto, CA) equipped with a Waters AccQ.Tag amino acid analysis column (3.9 mm × 150 mm, 4 µm. Waters, Milford, MA, USA) at 37 °C. The mobile phase flow rate was set at 1 mL/min and UV detection wavelength was 248 nm. Solvent A consisted of AccQ.Tag Eluent A (100 mL AccQ.Tag A concentrate + 1L ultra-pure water). Solvent B and C were acetonitrile and ultra-pure water respectively. Gradient conditions were shown in Table 5 - 2.

**Table 5 - 2:** Program of gradient conditions of free amino acids.

|  |  |  |  |
| --- | --- | --- | --- |
| Time (min) | A (%) | B (%) | C (%) |
| 0 | 100 | 0 | 0 |
| 0.5 | 99 | 1 | 0 |
| 18 | 95 | 5 | 0 |
| 19 | 91 | 9 | 0 |
| 29.5 | 83 | 17 | 0 |
| 35 | 0 | 60 | 40 |
| 38 | 100 | 0 | 0 |
| 47 | 100 | 0 | 0 |

Eluents: A, dilution of AccQ.Tag Eluent A with water (1:10 v/v); B, acetonitrile; C, ultra-pure water.

### 2.4.2. Determination of 5’-nucleotide analysis

5’-Nucletides were extracted and analyzed according to the method of (Hou, Liu, Xu, Zhou, & Li, 2018) with some modifications. 5.0 g of stewed pork sample was mixed 20 mL of 5% cold perchioric acid for 1 min and homogenized using an Ultra Turrax T10 (IKA, Königswinter Germany). The mixture was centrifuged at 3000 × *g* for 10 min, then the supernatant was filtered and adjusted to pH 6.5 by adding 1 M NaOH. All samples and eluents were filtered through 0.45 µm filtration membrane before analysis. The filtrate (10 µL) was injected into the Agilent 1200 HPLC fitted with Intersil ODS-3 column (250 mm × 4.6 mm; Waters) and UV detector (260 nm). Column temperature was set at 30 °C. Methanol (Eluent A) and 0.05% of phosphoric acid (Eluent B) were used as mobile phases at a flow rate of 1.0 mL/min. Gradient elution program was conducted as follows: 5% eluent A for 10 min, linear change to 15% eluent A for 5 min, then to 70% eluent A for 6 min, and finally to 5% eluent A for 4 min. The identification and quantification of nucleotides were assessed by comparison with the retention times and peak areas of nucleotide standards.

### 2.4.3. Calculation of equivalent umami concentration (EUC)

The EUC is defined as the concentration of the monosodium glutamate (MSG, mg/100 g) equivalent to the umami intensity given by a mixture of MSG and 5′-nucleotides and is calculated following equation: (Sun et al., 2014)

The EUC of the mixture in terms of g MSG/100 g, *ai* is the concentration (g/100 g) of each umami amino acid (Asp or Glu), *bi* is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1; Asp, 0.077), *aj* is the concentration (g/100 g) of each umami5′-nucleotide (5′-IMP, 5′-GMPor 5′-AMP), *bj* is the RUC for each umami 5′-nucleotide to 5′-IMP (5′-IMP, 1; 5′-GMP, 2.3; 5′-AMP, 0.18) and 1218 is a synergistic constant based on the concentration used.

### 2.4.4. Determination of fatty acid

Fatty acids were extracted from the freeze-dried pork samples with the chloroform-methanol according to the method reported by (Folch, Lees, & Stanley, 1957). Fatty acid methyl esters (FAMEs) were analyzed using a GC-7890 Agilent gas chromatography and a capillary column DB-5 MS (30 m ×0.25 mm × 0.25µm, Agilent Technologies, Santa Clara, USA). Individual fatty acid was identified by the comparison of retention time of FAME mixture (Sigma-Aldrich, Germany) and quantitated using the external standard method.

### 2.4.5. Electronic tongue analysis (E-tongue)

An electronic tongue system (ASTREE, Alpha MOS, France) consisting of 7 chemical sensors (AHS, SCS, ANS, CPS, NMS, CTS, PKS) with cross-selectivity was applied for taste measurements of stewed pork samples. To ensure the reliability and stability of the test data, the E-tongue was first self-tested, and the sensors were activated, calibrated and diagnosed. The sample solution was measured for 120 s, and the measurement data was recorded every 1s. The distilled water was used to clean the sensors for 30 s to ensure the stable potentials. Experiment was implemented at room temperature and each sample was analysed in triplicate.

## *2.5. Statistical analysis*

Statistical analysis was performed by SPSS 19.0 (IBM, Armonk, NY, USA), and one-way analysis of variance was used for the significant difference test (*P < 0.05*). The data were presented as the mean ± standard deviation. Principal component analysis (PCA) of E-nose and E-tongue were conducted using the software XLSTAT (2016) from Addinsoft (Barcelona, Spain).

# 3. Results and discussion

## *3.1. Volatile compounds profiling of stewed pork with different processing methods*

### 3.1.1. Volatile components analysis of stewed pork by GC-MS/O

The types and concentrations of volatile components in stewed pork with different processing techniques were detected by GC-MS/O. As shown in Table 5 - 3, there were about 60 volatile compounds were identified in different stewed pork samples, including aldehydes, alcohols, ketones, esters, hydrocarbons, ethers, phenols and heterocyclic compounds. These compounds classes agreed with the previous studies of pork flavour (Han et al., 2019; Zhao et al., 2017). Compared with the concentrations of aldehydes and hydrocarbons in traditional stewed pork (HS, HSE and HSEM), these compounds in high-temperature stewed pork (TS, TSE and TSEM) increased significantly (*P < 0.05*). On the contrary, the high-temperature stewed pork presented the lower concentration of ketones, ester, ethers and phenols than that observed in the traditional stewed pork. This might be due to the high temperature treatment of stewed pork increased the levels of lipid oxidation (Yang, Sun, Pan, Wang, & Cao, 2018). For the stewing pork with enzymatic hydrolysis and Maillard reaction, although there was no significant different (*P > 0.05*) in the concentration of volatile compounds in sample TSE and TSEM, the concentration of volatiles in sample HSEM were significantly higher (*P < 0.05*) than that in sample HSE. Which indicated that more complex compounds were produced by various chemical reaction, especially Maillard reaction, whereas these chemical reactions maybe reduce at lower temperature (Aaslyng & Meinert, 2017).

**Table 5 - 3:** The concentrations and types of volatile components in stewed pork with different processing methods.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Classes of components | Concentrations (μg/kg) (quantities) | | | | | |
| Traditional stewed pork | | | High-temperature stewed pork | | |
| TS | TSE | TSEM | HS | HSE | HSEM |
| Aldehydes | 4234.6c (9) | 2033.2d (5) | 4335.2c (12) | 5601.5b (9) | 4333.3c (8) | 8438.6a (16) |
| Alcohols | 1147.2c (8) | 884.6e (7) | 1196.3c (10) | 1055.0d (6) | 1411.0b (8) | 2213.0a (9) |
| Ketones | 101.4c (1) | 161.3a (2) | 147.1b (3) | N.D. | 81.6d (2) | 62.9e (1) |
| Esters | 32.5d (1) | 73.9a (1) | 39.9c (1) | N.D. | N.D. | 46.4b (1) |
| Hydrocarbons | 1008.9d (10) | 3134.7b (17) | 1602.1c (17) | 1585.7c (10) | 3556.1a (17) | 2900.2b (18) |
| Ethers | 1250.7c (4) | 2269.9a (4) | 1958.1b (4) | 999.7d (4) | 1967.6b (4) | 1256.8c (3) |
| Phenols | 474.1c (2) | 1298.0a (2) | 857.9b (2) | 154.1e (1) | 249.2d (2) | 260.0d (2) |
| Heterocyclic compounds | 262.6e (2) | 700.8b (3) | 334.0d (3) | 393.3c (3) | 419.1c (3) | 872.6a (3) |
| Total | 8512.0e (37) | 10556.4c (41) | 10470.6c (52) | 9789.3d (33) | 12017.9b (44) | 16050.5a (53) |

***Note:*** TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction. N.D., not detected.

### 3.1.2. Odour-active compounds of stewed pork

To estimate the contribution of individual compound to the overall aroma profile of different stewed pork, their odour activity values (OAVS) were calculated. The odour-active compounds (OAVS ≥ 1) of stewed pork were shown in Table 5 - 4. A total of 26 compounds were selected as odour active compounds in stewed pork. These volatile compounds of stewed pork belonged to 6 chemical classes: 13 aldehydes, 5 alcohols, 4 hydrocarbons, 2 ethers, 1 phenol and 1 furan. The hexanal (OAV at 139.6-491.8), heptanal (OAV at 28.8-99.4), octanal (OAV at 380.8-1166.4), nonanal (OAV at 781.8-2628.6), 1,8-cineole (OAV at 135.2-178.6), 1-octen-3-ol (OAV at 167.1-531.8), D-limonene (12.0-78.9), β-phellandrene (OAV at 10.2-32.0), estragole (OAV at 22.0-35.4), anethole (OAV at 55.9-125.0), eugenol (OAV at 21.7-171.6) and 2-pentylfuran (OAV at 19.9-96.4) with a higher level of OAVs had played a role in cooked pork, which had a great contribution to the whole flavour in all pork samples. In contrast, the 1-octanol (OAV at 0.6-2.0), styrene (OAV at 0.0-1.1) and naphthalene (OAV at 0.6-1.8) showed the lowest OAVs, indicating that they might have little contribution to pork flavour characteristic.

Additionally, some aldehydes, such as 2-methylbutanal (nutty odour), 3-methylbutanal (almond and nutty odour), (*E*)-2-octenal (green, nut and fatty odour), decanal (soap and orange peel odour), dodecanal (herbaceous and fatty odour) and (*E,E*)-2,4-decadienal (fried, wax and fatty odour) were detected in sample TSEM and HSEM. Which might be the main reason that stewed pork with the above methods promoted degradation reaction of amino acid, oxidation and degradation of lipid (Yang et al., 2018; Zou et al., 2018) to produce more important pork flavour. The OAVs of pentanal, hexanal, heptanal, octanal, nonanal, benzaldehyde and (*E*)-2-nonenal in sample HSEM were significantly higher (*P < 0.01*) than those of sample TS, TSE, TSEM, HS and HSE. This showed that sample HSEM had an advantage in contributing more pleasant fatty and grass aroma (Li et al., 2016) to the overall flavour.

For the identified alcohols, one linear alcohol (1-octanol) and three branched alcohols (1,8-cineole, 1-octen-3-ol and linalool) were mainly derived from the degradation of lipid (Pham et al., 2008). The lower odour threshold of alcohols (except for 1-octanol) were observed so they contribute significantly to mushroom, flower and mint note (Lorenzo, Carballo, & Franco, 2013) to the stewed meat products. The OAVs of alcohols in sample HSEM were the highest, indicating that the extent of lipid oxidation was greatest. In term of the dominated hydrocarbons, ethers and phenols, D-limonene, β-phellandrene, estragole, anethole and eugenol could be mainly formed from a small amount of lipid oxidation and various spices, such as anise, cardamun and other spiceries (Liu, Xu, & Zhou, 2007). The OAVs of these compounds with herbaceous and clove odour in sample TSE and HSE were significantly higher than those in sample TS, TSEM, HS and HSEM. 2-pentrylfuran, with the buttery odour, were usually an important volatile compound in cooked meat and meat product (Benet et al., 2015) and had the highest OAVs in sample HSEM, which could be due to linoleic acid oxidization (Aparicio, Morales, & Alonso, 1996). These founding showed that high-temperature stewed pork with enzymatic hydrolysis and Maillard reaction presented more aroma components.

**Table 5 - 4:** Odour-active compounds (OAVs > 1) in different stewed pork.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compounds | 1DB-Wax | 2Identification | 3Odour description;  4odour threshold (μg·kg-1) | Traditional stewed pork | | | High-temperature stewed pork | | | *p* value |
| TS | TSE | TSEM | HS | HSE | HSEM |
| 2-Methylbutanal | 906 | MS, RI, O | Nutty; 1 | N.D. | N.D. | 11.7±1.4 | N.D. | N.D. | N.D. | N.D. |
| 3-Methylbutanal | 910 | MS, RI, O | Almond, nutty; 4 | N.D. | N.D. | 12.8±2.1 | N.D. | N.D. | N.D. | N.D. |
| Pentanal | 969 | MS, RI, O | Almond, pungent; 9 | 13.5±1.2b | 6.1±0.3de | 5.2±0.2e | 11.1±0.7c | 7.0±0.4d | 17.0±0.5a | 0.000 |
| Hexanal | 1076 | MS, RI, O | Grass, fat; 4 | 426.7±7.2b | 176.9±5.0e | 139.6±7.0f | 353.0±2.8c | 215.6±6.9d | 491.8±4.2a | 0.000 |
| Heptanal | 1177 | MS, RI, O | Fat, citrus; 3 | 55.8±7.1cd | 28.8±1.3e | 68.0±1.9b | 62.4±0.4bc | 55.1±5.3d | 99.4±2.6a | 0.000 |
| Octanal | 1284 | MS, RI, O | Fat, lemon, green; 0.578 | 380.8±9.1e | 390.8±19.7e | 555.7±49.9d | 883.9±31.7b | 605.3±20.0c | 1166.4±9.3a | 0.000 |
| Nonanal | 1391 | MS, RI, O | Fat, citrus, green; 1 | 987.7±24.5e | 781.8±18.1f | 1446.7±63.2d | 2339.4±31.1b | 1560.9±28.7c | 2628.6±28.1a | 0.000 |
| (*E*)-2-Octenal | 1425 | MS, RI, O | Green, nut, fat; 3 | N.D. | N.D. | N.D. | N.D. | N.D. | 36.2±2.1 | N.D. |
| Decanal | 1490 | MS, RI, O | Soap, orange peel; 2 | N.D. | N.D. | N.D. | N.D. | N.D. | 76.8±5.8 | N.D. |
| Benzaldehyde | 1517 | MS, RI, O | Bitter almond, 41.7 | 8.7±0.5d | N.D. | 26.3±0.4a | 12.2±0.4c | 20.9±0.4b | 25.9±0.1a | 0.000 |
| (*E*)-2-Nonenal | 1534 | MS, RI, O | Cucumber, green; 1 | 63.1±12.9b | N.D. | 62.6±13.4b | 77.9±7.2b | N.D. | 133.0±35.3a | 0.008 |
| Dodecanal | 1708 | MS, RI, O | Herbaceous, fatty; 2 | N.D. | N.D. | N.D. | N.D. | N.D. | 22.8±1.1 | N.D. |
| (*E,E*)-2,4-Decadienal | 1808 | MS, RI, O | Fried, wax, fat; 0.07 | N.D. | N.D. | N.D. | N.D. | N.D. | 708.4±73.0 | N.D. |
| 1,8-Cineole | 1204 | MS, RI, O | Mint, sweet; 1 | 158.5±2.6b | 135.2±13.9c | 144.6±3.8c | 160.4±2.2b | 178.6±6.3a | 173.4±3.8a | 0.000 |
| 1-Octen-3-ol | 1445 | MS, RI, O | Mushroom; 2 | 268.2±8.9bc | 179.0±4.6d | 167.1±7.3d | 256.7±6.5c | 275.6±11.3b | 531.8±7.8a | 0.000 |
| Linalool | 1541 | MS, RI, O | Flower, lavender; 6 | 8.8±1.0c | 11.3±0.7b | 9.4±0.5c | 8.6±0.5c | 12.2±0.9ab | 13.3±1.1a | 0.000 |
| 1-Octanol | 1554 | MS, RI, O | Herbal, green; 110  green | 0.6±0.0c | 1.0±0.1c | 1.6±0.2b | 1.8±0.1b | 1.8±0.1b | 2.0±0.1a | 0.000 |
| (*E*)-2-Octen-1-ol | 1610 | MS, RI, O | Soap, plastic; 3 | 22.4±1.4cd | N.D. | 19.1±6.1d | 28.6±2.0b | 27.3±1.1bc | 46.4±2.3a | 0.000 |
| D-Limonene | 1187 | MS, RI, O | Citrus, mint; 10 | 14.4±1.5e | 40.3±0.4b | 12.0±0.7f | 32.9±0.7c | 78.9±0.7a | 17.5±1.7d | 0.000 |
| β-Phellandrene | 1197 | MS, RI, O | Turpentine, mint; 8  mint | 10.2±0.8e | 17.8±1.2c | 14.9±0.3d | 13.5±0.5d | 32.0±1.5a | 25.6±2.6b | 0.000 |
| Styrene | 1246 | MS, RI, O | Herbaceous, fatty; 65 | N.D. | 1.1±0.0b | 0.8±0.0c | N.D. | N.D. | 1.3±0.1a | 0.000 |
| Naphthalene | 1737 | MS, RI, O | Camphoric; 60 | 0.6±0.1d | 1.4±0.1b | 1.2±0.2c | 1.8±0.0a | 1.8±0.0a | 1.4±0.1b | 0.000 |
| Estragole | 1665 | MS, RI, O | Licorice, anise; 6 | 18.8±1.5e | 32.3±1.9b | 25.7±0.7c | 22.0±0.5d | 35.4±1.3a | 24.2±0.9cd | 0.000 |
| Anethole | 1822 | MS, RI, O | Rubber, paint; 15 | 71.2±2.1c | 125.0±0.7a | 113.0±2.1b | 55.9±0.8d | 112.3±1.4b | 71.2±1.2c | 0.000 |
| Eugenol | 2155 | MS, RI, O | Clove, honey; 7.1 | 61.4±2.0c | 171.6±3.3a | 110.2±1.7b | 21.7±0.8e | 30.2±0.3d | 31.3±2.1d | 0.000 |
| 2-Pentylfuran | 1222 | MS, RI, O | Green bean, butter; 6 | 36.9±2.0d | 21.7±0.4e | 19.9±0.2e | 55.8±0.2b | 45.4±2.9c | 96.4±2.3a | 0.000 |
| **Total** |  |  |  | 2608.3±71.3e | 2122±12.6f | 2968±118.4d | 4399.6±29.6b | 3296.3±20.6c | 6442.2±133.8a | 0.000 |

***Note:*** Each value is expressed as mean *±* SD; N.D. = not detected. a–f Different letters in the same row indicate that there is significant difference (*P <* 0.05, along the lines). TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

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

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

3 Odour thresholds were mainly obtained from online database, (http://www.flavornet.org, http://www.odour.org.uk).

4 Odour descriptions were mainly gathered from online database, (http://www.flavornet.org).

### 3.1.3. Volatile composition analysis of stewed pork using E-nose

The E-nose is very sensitive to the odour information of samples, minor changes in volatile compounds may cause differences of sensor response (Yang et al., 2016) and had been widely used in meat as an important method (Tian, Wang, & Cui, 2013). According to the response values of all sensors in Figure 5 - 2a, sensor W1W were most sensitive to the volatile compounds from the stewed pork, followed by sensors W1C, W5S, W3C, W6S, W5C, W2W and W3S, and finally sensors W2S and W1S. This result indicated that pork samples had large number of terpenes and sulphur containing organic compounds. The response values of W5S, W6S, W1S, W1W, W2S, W2W and W3S for sample HS, HSE and HSEM were significantly higher than that for sample TS, TSE and TSEM. However, the response values of W1C and W3C for sample TS, TSE and TSEM were significantly lower than those for sample HS, HSE and HSEM. The result demonstrated that the high-temperature stewing had a significant effect on the formation of furans, N- and S-containing compounds, the traditional stewing were more sensitive to aromatic compounds.

To evaluate the overall flavour characteristic of stewed pork samples, PCA was applied to analyse the E-noes data. As shown in Figure 5 – 2b, the showed a good discrimination from the different stewed pork since the first two PCs accounted for more than 95% of the total variances. PC1 explained 88.65% of the sample variance and PC2 explained only 8.46%. Therefore, the major variation resulting from PC1 were employed to distinguish the differences among stewed pork sample. The traditional stewed pork samples (TS, TSE and TSEM) and high-temperature stewed pork samples (HS, HSE and HSEM) were located on the negative and positive axis respectively and could be divided two different groups on PC1. This result illustrated that the stewed pork from traditional and high-temperature processing method had significantly different flavour profiles, sample TS, TSE and TSEM had similar aroma compositions, and sample HS, HSE and HSEM were the same. Sample TS, TSE and TSEM on PC1 were highly associated with sensors W1C and W3C, and sample HS, HSE and HSEM on PC1 were highly related to sensors W6S, W1W, W2W, W3S, W2S, W5S and W1S. The stewed pork samples on PC2 were depended on W5C, which was sensitive to alkane compounds. Due to the little variance contribution rate of PC2, the alkane compounds had no important influence on the odour of stewed pork. Corresponds to the result of Fig, 5.1a, aromatic compounds, ammonia, nitrogen oxides, broad alcohols, sulphur organic compounds, terpenes and organic sulphides were on the dominant position of stewed pork odour.

**G/G0**

**(a)**

**(b)**

**Figure 5 - 2:** Response values of ten sensors **(a)** and PCA chart **(b)** of volatile flavour compounds in stewed pork with E-nose. The sensory of 10 chemical sensors are W1C (aromatic), W5S (broad-range), W3C (aromatic), W6S (hydrogen), W5C (arom-aliph), W1S (broad-methane), W1W (sulfur-organic), W2S (broad-alcohol), W2W (sulph-chlor) and W3S (methane-aliph). TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

## *3.2. Taste compounds profiling of stewed pork with different processing methods*

### 3.2.1. FAA analysis of stewed pork

The concentrations of FAA of stewed pork with different processing methods were showed in Table 5 – 5. A total of 16 amino acids were detected in stewed pork samples, which were divided into umami amino acids (UAAs), sweet amino acids (SAAs), bitter amino acids (BAAs) and other amino acids. Among them, the content of SAAs (112.9-148.2 mg/100g) was highest in all stewed pork, indicating that the SAAs were predominant in the stewed pork. The observed results of SAAs in the present study were consistent with those reported in stewed pork rib broth (Hou et al., 2018). The UAAs, SAAs and BAAs showed higher values in sample HS, HSE and HSEM with heat treatment than in traditional stewed pork (TS, TSE and TSEM). This may be because the higher temperature applied during meat processing could generate a major release of free amino acids (Diaz, Fernandez, De Fernando, de la Hoz, & Ordoñez, 1997).

The total FAA presented higher contents in sample HSE than in sample HS, and samples HSEM had lowest contents of total FAAs. It indicated that the flavorzyme hydrolysed proteins to produce more FAA (Xu et al., 2018), while the decrease in FAAs after adding xylose was related to the formation of volatile compounds by Maillard reaction (Song et al., 2019). No significant difference of total FAA has been found in samples TS, TSE and TSEM. Among the FAA, the content of Asp in sample HSEM was highest, samples HS and HSE had the highest content of Glu, which could give stewed pork a strong umami taste. The content of SAAs (Ser, Gly, Thr and Ala) in sample HSEM were less than that of the other two samples (HS and HSE). Sample HS and HSE showed a higher content of BAAs except for His and Arg than the other stewed pork samples. The bitterness produced BAAs could be masked by sweet and umami substance such as Asp, Glu, Ser, Gly, Thr and Ala. In order to determine the contribution of free amino acids to the taste of stewed pork, the taste threshold was introduced (Table 5.5). The taste activity values (TAVs) of each amino acid were calculated (Liu, Xia, Wang, & Chen, 2019) by taste threshold. Although it can be seen that the TAVs of free amino acids in stewed pork was < 1 to less contributed taste, the umami taste of stewed pork might increase by the synergistic interaction between FAAs and nucleotides.

**Table 5 - 5:** Free amino acid contents (mg/100g) and taste threshold of stewed pork with different processing methods.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Free amino acid | Traditional stewed pork | | | High-temperature stewed pork | | | P value | Taste threshold (mg/100g) |
| TS | TSE | TSEM | HS | HSE | HSEM |
| Asp | 2.4±0.1c | 2.8±0.1bc | 3.3±0.4ab | 2.5±0.5c | 2.8±0.3bc | 3.7±0.3a | 0.002 | 100 |
| Glu | 13.9±0.5a | 11.5±0.8b | 13.0±0.2ab | 15.1±1.0a | 14.4±0.3a | 11.0±2.4b | 0.004 | 30 |
| ƩUAA | 16.3±0.4ab | 14.2±0.8b | 16.3±0.2ab | 17.6±1.0a | 17.2±0.5a | 14.7±2.7b | 0.030 |  |
| Ser | 5.3±0.2b | 2.9±0.3c | 6.1±0.3b | 8.3±0.9a | 8.1±0.3a | 2.6±0.1c | 0.000 | 150 |
| Gly | 7.1±0.5bc | 6.2±0.3c | 7.5±0.2b | 9.5±0.6a | 7.6±0.1b | 6.3±1.0c | 0.000 | 130 |
| Thr | 96.0±3.5bc | 113.6±7.9a | 88.5±6.3c | 115.9±8.1a | 106.3±1.8ab | 97.2±2.7bc | 0.000 | 260 |
| Ala | 5.6±0.5e | 8.0±0.7d | 9.7±0.1c | 14.6±1.5a | 12.1±1.3b | 6.8±0.6de | 0.000 | 60 |
| ƩSAA | 114.1±4.7c | 130.7±8.7b | 111.7±6.2c | 148.2±11.2a | 134.0±3.1b | 112.9±4.2c | 0.000 |  |
| His | 18.0±0.7c | 16.9±0.1d | 17.7±0.1c | 18.4±0.2bc | 18.8±0.0b | 23.1±0.6a | 0.000 | 20 |
| Arg | 27.2±1.2a | 18.1±1.1d | 24.8±1.4bc | 26.5±1.8ab | 23.5±0.3c | 23.1±0.6c | 0.000 | 50 |
| Val | 4.8±0.1cd | 5.3±0.2bc | 5.5±0.3b | 7.1±0.6a | 7.5±0.2a | 4.6±0.1d | 0.000 | 40 |
| Met | 11.5±0.1a | 10.9±0.1b | 11.9±0.1a | 12.1±0.7a | 11.8±0.0a | 10.5±0.0b | 0.000 | 190 |
| Ile | 8.1±0.2c | 7.8±0.3c | 9.3±0.4b | 10.4±0.5a | 10.6±0.1a | 7.7±0.1c | 0.000 | 90 |
| Leu | 4.4±0.3b | 1.1±0.1c | N.D. | 5.7±0.6a | 5.2±0.2a | 0.1±0.0d | 0.000 | 30 |
| Phe | 21.1±0.6bc | 20.6±0.6c | 21.5±0.3ab | 21.5±0.5ab | 22.0±0.1a | 20.8±0.0bc | 0.011 | 90 |
| ƩBAA | 95.1±1.5b | 80.6±1.4d | 90.7±2.4c | 101.8±3.8a | 99.5±0.8a | 89.9±1.4c | 0.000 |  |
| Pro | 5.7±0.1c | 5.7±0.2c | 5.8±0.2c | 7.3±0.2b | 8.5±0.1a | 5.7±0.2c | 0.000 | 300 |
| Tyr | 5.8±0.0e | 15.7±1.1bc | 17.2±1.3b | 12.3±1.0d | 41.2±2.8a | 13.5±0.6cd | 0.000 | / |
| Lys | 0.5±0.2d | 0.1±0.0d | 1.3±0.4c | 3.4±0.5a | 2.4±0.2b | 0.2±0.0d | 0.000 | 50 |
| Total | 237.5±6.8b | 247.0±10.5b | 243.1±10.2b | 290.7±16.5a | 302.8±6.5a | 236.8±3.5b | 0.000 |  |

***Note:*** Each value is expressed as mean ± SD. a–e Different letters in the same row indicate that there is significant difference (*P < 0.05*, along the lines). UAA: Umami amino acids, SAA: Sweet amino acids, BAA: Bitter amino acids, FAA: Free amino acids. N.D., not detectable. TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

### 3.2.2. 5’-Nucleotide and EUC analysis of stewed pork

As can be seen in Table 5 - 6, 5’-nucleotides (AMP, GMP and IMP) of traditional stewed pork (TS, TSE and TSEM) exhibited higher contents compared with that in high-temperature stewed pork (HS, HSE and HSEM). This might be one possible reason that the higher temperature in steaming step of stewed pork could accelerated the degradation of nucleotides to produce a better flavour (Dinesh D Jayasena et al., 2015). Whether traditional stewed pork (TS, TSE and TSEM) or high-temperature stewed pork (HS, HSE and HSEM) with the addition of flavorzyme and flavour precursors (xylose, cysteine and thiamine) showed an increasing trend in the contents of AMP, GMP and IMP, indicating enzymatic hydrolysis and Maillard reaction were prone to promote the formation of flavour nucleotides. Similarly, the total 5’-nucleotides contents also had significantly increased in traditional stewing groups (TS, TSE and TSEM) or high-temperature stewing groups (HS, HSE and HSEM).

IMP was the most predominant flavour-contributing 5’-nucleotide in stewed pork and is known to impart a pleasant taste (Yue, Zhang, Jin, Deng, & Zhao, 2016). The interaction of IMP with some sweet amino acids like Ser, Gly and ALa has been shown to contribute to intensify umami taste (Kawai, Okiyama, & Ueda, 2002). According to taste threshold of nucleotides, the TAVs of IMP were much greater than 1 to provide more umami taste. As for AMP and GMP, the contents of sample HS were lowest and those of sample TSEM were highest. Although the TAVs of AMP and GMP in the pork samples were lower than 1, GMP is a stronger flavour enhancer contributing to a meaty flavour (Yue et al., 2016) and the synergistic interaction of between IMP and AMP in eliciting umami taste should be considered (Fuke, & Ueda, 1996). Due to the synergistic effect of flavour nucleotides and MSG-like components (Glu, and Asp), it might greatly increase the umami taste in marinated chicken (Li et al., 2016). Therefore, EUC values were suggested to evaluate the umami taste of stewed pork. Sample TSE, TSEM, HSE and HSEM had the higher level of EUC value (Table 5 - 6). Overall, the stewed pork with enzymatic hydrolysis and Maillard reaction had a more umami taste than other stewed pork.

**Table 5 - 6:** Nucleotide contents (mg/100g), taste threshold and EUC of stewed pork with different processing methods.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Nucleotides | Traditional stewed pork | | | High-temperature stewed pork | | | P value | Taste threshold (mg/100g) |
| TS | TSE | TSEM | HS | HSE | HSEM |
| 5’-AMP | 20.2±0.3c | 25.5±2.1a | 18.8±0.4c | 19.3±0.4c | 22.7±1.2b | 27.0±0.3a | 0.000 | 50 |
| 5’-GMP | 2.6±0.0b | 2.7±0.0ab | 2.9±0.0a | 1.6±0.0d | 2.0±0.2c | 1.9±0.2c | 0.000 | 12.5 |
| 5’-IMP | 107.6±1.2d | 116.1±3.4c | 149.4±2.1a | 77.7±1.1e | 116.1±2.7c | 126.9±1.1b | 0.000 | 25 |
| 1Flavor 5’-nucleotide | 130.4±1.0d | 144.3±5.5c | 171.1±2.5a | 98.6±1.5e | 140.8±3.1c | 155.7±1.1b | 0.000 |  |
| 2EUC (g MSG/100 g) | 1.9±0.0bc | 2.2±0.0ab | 2.3±0.1a | 1.6±0.1c | 2.2±0.1ab | 1.9±0.4bc | 0.006 |  |

***Note:*** Each value is expressed as mean ± SD (n = 3). Means with different superscripts in the same row indicate significant difference (*P < 0.05*). 1 Flavor nucleotides = 5'-IMP+5'-GMP+5'-AMP. 2 The equivalent umami concentration (EUC, g monosodium glutamate (MSG) per 100 g) represents the concentration of MSG equivalent to the umami intensity given by the mixture of MSG and the 5’-nucleotide. TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

### 3.2.3. Fatty acid composition of stewed pork

Fatty acid composition in meat is important for consumers due to their major contributions to meat flavour (Aaslyng & Meinert, 2017). As shown in Table 5 - 7, it was observed that 9 fatty acid were present in stewed pork with different processing methods. The main fatty acids detected in stewed pork samples were C16:0 (palmitic acid), C18:0 (stearic acid), C17:1 (ginkgolic acid), C18:1 (oleic acid), C18:2 (linoleic acid) and C20:4 (arachidonic acid) which were approximately 94% of the total fatty acids. The single fatty acids or total saturated fatty acids (SFAs), total monounsaturated fatty acids (MUFAs) and total polyunsaturated fatty acids (PUFAs) changed significantly (*P ≤ 0.001*) in high-tempreature stewed pork (HS, HSE and HSEM) compared with traditional stewed pork (TS, TSE and TSEM), however C14:0 was not markedly different in all stewed pork samples. For traditional stewed pork, sample TSEM had significantly lower (*P ≤ 0.05*) SFA and MUFA than sample TS and TSE. On the contrary, PUFA increased significantly (*P ≤ 0.05*) in sample TSEM which was probably because the higher level of Maillard reaction products (Hwang, Kim, Woo, Lee, & Jeong, 2011) inhibit autoxidation of PUFA during processing. The concentrations of SFA, MUFA, PUFA in high-temperature stewed pork decreased significantly (*P < 0.05*), when flavormyze, xylose, cysteine and thiamine was added. It may be due to the thermally induced reactions between fatty acid oxidation products, enzymatic hydrolysis products and Maillard reaction precursors could form a large number of volatile compounds. From the above results, it can be concluded that the stewed pork combined with both enzymatic hydrolysis and Maillard reaction increased the possibility of fatty acid oxidation.

**Table 5 - 7:** Concentrations (mg/kg) of fatty acid in stewed pork with different processing methods.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Fatty acids | Traditional stewed pork | | | High-temperature stewed pork | | | P value |
| TS | TSE | TSEM | HS | HSE | HSEM |
| C14:0 | 85.1±0.6a | 82.5±6.3ab | 80.9±1.6ab | 84.0±2.8ab | 82.9±5.8ab | 74.2±9.5b | 0.241 |
| C16:0 | 787.9±14.7a | 746.4±43.4ab | 699.2±16.7bc | 685.3±37.7cd | 638.7±30.2d | 579.4±20.3e | 0.000 |
| C18:0 | 798.1±14.6a | 774.9±6.1a | 675.7±9.6b | 667.9±4.7b | 640.2±33.8b | 558.3±28.8c | 0.000 |
| C16:1 | 262.8±2.8a | 260.2±7.4a | 245.1±10.3b | 295.5±6.6b | 228.2±20.4c | 211.0±6.1d | 0.000 |
| C17:1 | 804.7±32.6bc | 735.7±13b | 676.6±4.7b | 818.9±18.4a | 690.1±7.4cd | 545.9±7.4d | 0.000 |
| C18:1 | 889.1±55.3c | 832.8±27.5b | 754.7±33.8a | 1234.0±162.2a | 952.2±52.9c | 940.6±61.7d | 0.000 |
| C18:2 | 2664.4±146.4c | 2990.3±37.3bc | 3058.8±10.7bc | 2870.4±28.7a | 2450.5±18.8b | 2126.8±70.3b | 0.000 |
| C20:4 | 1348.6±108.1bc | 1407.2±59.7bc | 1547.9±58.7bc | 1391.1±33.5a | 1250.0±33.1b | 1074.6±72.4c | 0.000 |
| C20:5 | 117.2±5.3a | 122.5±6.8ab | 132.2±5.8a | 119.1±3.7b | 110.0±6.2d | 106.2±2.0e | 0.001 |
| SFA | 1671.0±18.7bc | 1603.8±34.3b | 1455.8±10.5a | 1437.2±39.6b | 1361.8±58.2b | 1211.9±48.5d | 0.000 |
| MUFA | 1956.5±60.9b | 1828.7±36.1bc | 1676.4±47.5d | 2348.4±148.6a | 1870.5±80.5cd | 1697.5±59.7cd | 0.000 |
| PUFA | 4130.1±245.3b | 4520.0±30.2b | 4739.0±44.0a | 4380.6±6.1b | 3810.5±9.9c | 3307.6±88.6e | 0.000 |
| Total | 7757.7±225.6b | 7952.5±100.5ab | 7871.2±83.7b | 8166.3±110.3a | 7042.8±31.8c | 6217.1±168.3d | 0.000 |

***Note:*** SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

### 3.2.4. Taste composition analysis of stewed pork by E-tongue

PCA was applied to provide an overview of taste compounds of stewed pork samples using E-tongue data. As shown in Figure 5 - 3, the first two PCs explained 59.86% and 29.86% of the data variance, respectively. The total contribution rate was over 85%, which showed that most of information can reflect the overall characteristics of the pork samples. Six different stewed pork samples were clearly divided into three groups (HS; TS and TSEM; HSEM, HSE and TSE). The sample dots of HS, HSEM, HSE and TSE were located on the positive side of PC1, whereas sample HS, HSEM, HSE and TSE were on the opposite side. This indicated that the taste characteristics of sample TS and TSEM were significantly different from those of sample HS, HSEM, HSE and TSE. The sample points of HSEM, HSE and TSE were clustered together, and sample TS and TSEM were close each other, which meant that there was a similar taste profile. The sample dots of HS were distributed separately in the upper left side, which were described as sourness and bitterness because of their short distance to sensor AHS and SCS. Sample TS and TSEM were responsible for sensor CTS (to detect salty substances) and sensor PKS, ANS and NMS were lowly relevant to the stewed pork samples.

**Figure 5 - 3:** PCA score plot of E-tongue data for stewed pork with different processing methods. TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction. Taste sensor: AHS (to detect sour taste), SCS (to detect bitterness), PKS (to detect complex taste), ANS (to detect sweetness), NMS (to detect umami taste), CPS (to detect complex taste), CTS (to detect salty taste).

# 4. Conclusions

In this present study, the types and contents of volatile compounds in high-temperature stewed pork (HS, HSE and HSEM) were higher significantly (*P < 0.05*) than those of traditional stewed pork (TS, TSE and TSEM) in particular of aldehydes. For high-temperature stewed pork, sample HSEM showed the most abundant flavour compounds such as aldehydes, alcohols and heterocyclic compounds. Most of odour active compounds in sample HSEM had the highest OAVs, which could contribute more typical aroma to stewed pork. All stewed pork samples were clearly divided into two groups, including traditional stewed samples (TS, TSE and TSEM) and high-temperature stewed samples (HS, HSE and HSEM), which indicated that the volatile composition of two groups of pork samples were significantly different. Sample HS, HSE and HSEM had the higher contents of UAAs, SAAs and BAAs than sample TS, TSE and TSEM, because the high temperature promote the major release of FAA. The contents of 5’-nucleotides (AMP, GMP and IMP) in high-temperature stewed pork (HS, HSE and HSEM) showed lower level due to heat-induced decomposition of nucleotides to produce more flavour compounds. The contents of fatty acids in stewed pork samples decreased significantly (*P < 0.05*), when flavormyze, xylose, cysteine and thiamine was added. It can be concluded that high-temperature stewed pork (HS, HSE and HSEM) improve the taste and odour characteristic, of which high-temperature stewed pork with enzymatic hydrolysis and Maillard reaction was particularly prominent in the formation of odour compounds.

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**6**

Chapter Ⅵ. General discussion, conclusions and perspective

# 1. General discussion

## *1.1. Analysis of number of experiment samples*

In order to understand the flavour compounds in the stewed pork, four brands of stewed pork with typical characteristics in China were selected for research. For each brand, three different lot numbers were collected. Although there were few stewed pork samples, the pork samples from different batches were random and the overall flavour and volatile compounds of stewed pork could be analysed comprehensively and accurately. The larger the amount of stewed pork samples, the more favourable it is for the research on the flavour of stewed pork. To study the influence of different pork varieties on the flavour of boiled pork, Chinese local pigs (SMX, TB) and foreign hybrid pigs (DLY) were selected for this subject. TB, SMX and DLY pork samples (n = 6 for each breed) from the fore and hind leg muscle were collected, and the same muscle gathered from two individual pigs of the same breed was combined as one sample (n = 3 for each muscle) for volatiles analysis. Six pork samples of each variety were used for data analysis, a large data form the volatile compounds in boiled pork could be obtained, and the treatments of these boiled pork sample might be cumbersome. Which would bring some errors to the experimental data. Two samples of the same pork variety would be mixed to reduce the cost of sample operation. It was also better to obtain the information of overall flavour from the pork samples. We have also studied the influence of different spice formulas on the flavour of stewed pork. The raw material of the experiment was hind leg meat of pig and a total of 42 pieces of hind leg muscles are divided into seven groups. Each group of pork samples was processed with different spice recipes. The quantity of these samples was enough to discuss the flavour of stewed pork from different seasoning recipes. In additional, the influence of different processing methods on the flavour of stewed pork was investigated. Each processing method was repeated three times to ensure the accuracy of the test. In our previous research, our research team used the amounts of data in label-free quantitative proteomics analysis of different pork (Mi, Shang, Li, et al., 2019) and mineral profiling of Taihe black-boned silky fowl (*gallus gallus domesticus brisson*) muscles (Mi, Shang, Jia, Zhang, & Fan, 2019) to support the research experiments, however, it was often used to achieve the experimental objective for the study of flavour of stewed pork by a small amount of repeated tests. There were also reports that the preparation and flavour determination experiments of braised pork were carried out in triplicate (Song et al., 2019) and the results were showed as mean values. Each sample of stewed spice beef were repeated in parallel three times, which were performed the analysis of volatile compounds by GC-MS coupled with E-nose (Gong et al., 2017). The group of marinated pork samples was carried out in six replicates and assess the effects of ultrasonic assisted cooking on the chemical profiles of taste and flavour (Zou et al., 2018). The repetition times of the above samples are similar to the repetition times of the stewed pork samples in this subject, which can be well used for the detection of meat flavour.

## *1.2. Types of volatile compounds and odour-active compounds*

The traditional stewed pork is popular by consumers in China due to the unique and desirable flavour (Yang et al., 2019). Flavour is one of the most important eating quality of various meat products (Kosowska, Majcher, & Fortuna, 2017). As far we know, over 1000 volatile compounds have been detected in meat and meat products (Mottram, 1998), including aldehydes, alcohols, hydrocarbons, ethers, ketones, furans, N- and S- containing compounds (Marušić, Vidaček, Janči, Petrak, & Medić, 2014; Qin, Cai, Zhang, Liu, & Lai, 2019). It has been found that aldehydes, alcohols and heterocyclic compounds were identified to mainly contribute to the overall flavour of stewed pork. Similar results were also reported in cooked meat (Maughan, & Martini, 2012; Soncin, Chiesa, Cantoni, & Biondi, 2007). Although the stewed pork presented the higher amounts and contents of hydrocarbons, there was a litter contribution to the integral flavour due to their high threshold values (Han et al., 2019). Therefore, the aldehydes, alcohols and heterocyclic compounds played the major roles in the stewed pork samples.

Aldehydes are the secondary products and produced primarily by lipid oxidation and Strecker degradation products of amino acids ([Li et al., 2016](#_ENREF_31); [Zhao et al., 2017](#_ENREF_70)). Which are also known to be major contributors to the unique flavour of cooked pork due to their low odour threshold ([Lorenzo & Fonseca, 2014](#_ENREF_37)). Obviously, the content and number of aldehydes were the most abundant of stewed pork samples in different chapters. This indicated that aldehydes contributed significantly to the overall flavour of meat products ([Li et al., 2016](#_ENREF_31); [Petričević, Radovčić, Lukić, Listeš, & Medić, 2018](#_ENREF_47)). It can be also found that seven compounds, including four alkenals (hexanal, heptanal, nonanal and octanal), two alkadienals ((*E*)-2-octenal and (*E*)-2-nonenal), as well as one phenyl-containing aldehyde (benzaldehyde) were common volatiles in all stewed pork from different brands, breeds of pigs, seasoning recipes and processing methods. Among them, the alkenals and alkadienals could be generated from the degradation of unsaturated fat acids ([Jayasena, Ahn, Nam, & Jo, 2013](#_ENREF_27)), and the phenyl-containing aldehyde were derived from Strecker degradation ([Gu, Wang, Tao, & Wu, 2013](#_ENREF_22)). Hexanal is regarded as a measure of lipid peroxidation (Frankel, Hu, & Tappel, 1989). Moreover, the content of hexanal with grassy notes in fresh pork significantly (*P < 0.01*) lower than that in pork after stewing, indicating that the extent of lipid oxidation in stewed pork than fresh pork. Besides, the contents of some aldehydes, such as octanal, (*E*)-2-octenal and (*E*)-2-nonenal, was significantly decreased when when adding seasoning (spices, soy sauce, sugar and cooking wine) to the stewed pork. It showed that seasoing addtion has an inhibitory effect on the formation of aldehydes in stewed pork samples. This discovery was supported with ([Mancini, Paci, Dal Bosco, Mattioli, & Preziuso, 2019](#_ENREF_39)), who has reported that the ginger powder could express invreasing the antioxidation capacity and reducing lipid oxidation of burgers. Benzaldehyde could present fruity and floral notes and be generated from the Chinese different spices ([Wang, Hong, Ke, Hu, & Chen, 2017](#_ENREF_61)), including fennel, aniseed and cinnamon respectively. In addition, a small amount of long-chain aldehyde is found in stewed pork samples. These compounds are usually formed by oxidation of aldehydes ([Han, Zhang, Fauconnier, & Mi, 2019](#_ENREF_24)) and contribute less to the overall flavour of stewed pork.

Alcohols are another main class of volatile compounds, which could be associated with the oxidative decomposition of lipid ([Toldrá, 2017](#_ENREF_59)) and spices ([Gong et al., 2017](#_ENREF_21)). In terms of the number of alcohol compounds, the stewed pork from spice recipes were significantly hihger that that from different brands, breeds and processing technology. These compounds are classified into two major categories of long-chain and short-chain alcohols. Compared with short straight chain alcohols, long chain alcohols are considered to have more contributions to the aroma of meat products due to their lower odour thresholds ([Li et al., 2016](#_ENREF_31)). Four alcohols were detected simultaneously in this study, which are 1,8-cineole, 1-octen-3-ol, 1-octanol and (*E*)-2-octen-1-ol. The thermal processing of pork had significantly higher level of 1-octen-3-ol, 1-octanol and (*E*)-2-octen-1-ol. This may be because high temperature promotes the reaction rate of lipid oxidation. The content of 1-octen-3-ol, with mushroom notes, increased significantly when the spices were added in the stewed pork. This result found that 1-octen-3-ol was mainly formed by different spices and it was also detected in the fish products ([Zhou, Chong, Ding, Gu, & Liu, 2016](#_ENREF_71)). For four alcohols identified above, their odour thresholds were lower than their concentration in stewed pork, which indicated they provided more contribution to the whole flavor profile of stewed pork samples. While, some alcohols such as terpinen-4-ol, α-terpineol and 2-phenylethanol had very high thresholds, which indicated that they were not the main flavor substances but exerted a synergistic influence on the total flavor.

Furans, nitrogen and sulphur-containing compounds are well known as important heterocyclic compounds in meat products ([Wang et al., 2018](#_ENREF_62)). Furans refer to a group of heterocyclic compounds that were structurally characterized with the oxygen atom in the ring. It was reported that the formation of furans might mainly come from three pathways: the dehydration of carbohydrates, the Amadori rearrangement procedure ([Giri, Osako, & Ohshima, 2010](#_ENREF_20)) and oxidation of fatty acids ([Taylor & Mottram, 1990](#_ENREF_57)). Among the identified furans, the most important compounds were 2-pentylfuran and 2-ethylfuran in cooked meat ([Benet et al., 2015](#_ENREF_5)). 2-pentylfuran and 2-ethylfuran were detected in all stewed pork samples from the brands, breeds of pigs, seasoning recipes and processing methods. Which could derive from the autoxidation of linoleic acid with a fruity and butter odour ([Aparicio, Morales, & Alonso, 1996](#_ENREF_3)). In addition, 2-furanmethanol usually have caramel odours in meat products and could be produced by Maillard reaction ([Aaslyng & Meinert, 2017](#_ENREF_1)). The nitrogen and sulphur-containing compounds mainly derived from the catabolism of proteins, free amino acids and nucleic acids ([Chen, Song, & Ma, 2009](#_ENREF_8)). Only 2-acetylpyrazine were identified in all stewed pork samples and it was also found that the amounts and contents of 2-acetylthiazole in TB were greater than in DLY and SMX. This analysis indicated that in boiled pork in TB are regarded as the major contributor to the caramel and roasted flavours. For the research of stewed pork from different spice recipes, 3-(4-methyl-3-pentenyl)-furan, furfural, 2-furanmethanol, pyridine, 2-acetylpyrrole and dimethyl trisulfide displayed significantly higher levels (*P < 0.01*) in SP5 and SP6 than those in other groups, which indicated that the addition of sugar and cooking wine could promote the Maillard reaction. Regarding methanethiol and dimethyl disulphide were significantly lower in SP1 than that in SP2, which indicated that salt-treated stewed pork was more conducive to the production of sulphur-containing compounds. This result was consistent with that reported by (Liu, Xu, Ouyang, & Zhou, 2006) who found that the levels of sulphur-containing compounds in Nanjing water-boiled salted duck were markedly higher than those in control samples. It also has been reported that these compounds might originate from Maillard reaction and degradation of sulphur amino acids (free, peptidic and proteinic amino acids), thiamine or glutathione ([Girard & Durance, 2000](#_ENREF_19)). Hydrocarbons had few effects on the aromatic profiles of meat products due to their high odour thresholds ([Qi, Liu, Zhou, & Xu, 2017](#_ENREF_49)) and could be derived from alkyl radicals via lipid auto-oxidation processes ([Fu, Xu, & Wang, 2009](#_ENREF_18)). Other types of volatiles including ketones, esters and phenols are also considered as a flavor auxiliary of the stewed pork, although they have relatively high thresholds.

The meat is cooked at high temperature by frying, boiling and grilling. While there is the positive correlation between the intakes of toxic Maillard reaction products like heterocyclic amines (HCAs) from foods and increased risk of human cancer ([Cross et al., 2010](#_ENREF_11); [Lin et al., 2010](#_ENREF_33)). However, some other studies have not showed any correlation between HCAs and cancer risk ([Ollberding, Wilkens, Henderson, Kolonel, & Le Marchand, 2012](#_ENREF_44); [Sander, Linseisen, & Rohrmann, 2011](#_ENREF_53)). It has been found that compared with other methods, the roasting of meat generated less of HCAs ([Sinha et al., 1998](#_ENREF_54)) and the commercially cooked meat contained low amounts of HCAs ([Tikkanen, Latva-Kala, & Heiniö, 1996](#_ENREF_58)). It also has been reported that boiling and microwave cooking ([Liao, Wang, Zhang, Xu, & Zhou, 2012](#_ENREF_32)) were the most appropriate methods to process duck for the formation of Maillard reaction products. In our study, the stewed pork from four brands were obtained from the local supermarket. Which belongs to the commercially cooked with low level of HCAs. Moreover, the experimental pork was stewed at low temperature would not be conducive to HCAs and the new processing technology of the stewed pork was mainly the roasting step that decreased significantly the content of HCAs. In summary, toxic Maillard reaction products in the stewed pork samples could be ignored.

As far we know, among many flavour compounds, only a small part of the compounds have potential contribution to meat products (Gu, Wang, Tao, & Wu, 2013; Song & Liu, 2018). To explore the contribution of these volatile compounds to aroma profile of meat products, the OAVs were always taken into consider (Chen, Song, & Ma, 2009). The OAVs could be calculated by dividing the contents of volatile compounds by their thresholds in a matrix (Frauendorfer & Schieberle, 2006). The study has reported that pentanal, hexanal, heptanal, octanal, (E)-2-octenal, nonanal, decanal and (E,E)-2,4-decadienal, 1-pentanol, 1-octen-3-ol and 2-n-butylfuran were the primary odour compounds (OAV ≥ 10) in the stewed yellow feather chicken (Qi et al., 2018), indicating that aldehydes were the most important odour compounds in cooked meat. In our study, the hexanal (OAV at 3.6–20.3), octanal (OAV at 30.3–47.5), nonanal (OAV at 68.6–166.3), 1,8-cineole (OAV at 36.4–133.3), anethole (OAV at 5.9–28.3) and 2-pentylfuran (OAV at 3.5–29.7) were the key odour-active compounds in stewed pork. This result showed that six odour-active compounds also had more contribution to stewed pork samples. The above two results indicated that the main flavour compounds in different types of stewed pork (stewed chicken and pork) were also significantly different.

## *1.3. Analysis methods of volatile compounds in stewed pork*

The GC-MS/O technique, a combination of GC-MS and GC-O, is an effective tool to study food flavour (Song & Liu, 2018) and has been widely used for identification of volatile compounds in meat products, such as cured pork leg (Pu et al., 2020), roast duck (H. Liu et al., 2019), lamb meat (Bueno et al., 2011). The qualitative and quantitative analysis of individual compounds could be well performed by GC-MS/O, however the overall flavour of stewed meat could not be evaluated. For this reason, the E-nose technology was applied to determine the integral aroma composition of stewed pork samples in this study. The application of the E-nose has been reported in the discrimination and the flavour assessment of wines (Liu et al., 2012), in the prediction of the degree of decay in fruits (Hui et al., 2012; Pan, Zhang, Zhu, Mao, & Tu, 2014) and the classification of milk flavours (Bei, Xu, & Sun, 2010). The GC-MS/O and E-nose were simultaneously employed to obtain reliable data on the odorant composition of stewed pork.

Nowadays, there were many analysis techniques for volatile flavour compounds in food. Among them, the GC × GC-TOFMS based on multivariate data analysis is a powerful technique for volatiles fingerprinting profiling of flavour compounds with low concentration, complex composition and high volatility (Adahchour, Beens, & Brinkman, 2008; Chin & Marriott, 2014; Cordero, Kiefl, Schieberle, Reichenbach, & Bicchi, 2015). Compared to one dimensional system, GC × GC could provide higher separation power due to the different separation selectivity of two series chromatographic dimensions (Huang et al., 2019). In our study, more volatiles (e.g. aldehydes, alcohols, ketones and furan, N- or S-containing compounds) were detected by GC × GC-TOFMS, ethers and acids were not identified by GC-MS/O. It may be because two different polar and non-polar capillary columns (DB-WAX and DB-17HT) were connected together to analyse the volatiles. This result could be well explained by (Wang et al., 2018). Furthermore, some long-chain aldehydes and hydrocarbons, such as β-cyclocitral, tetradecanal, hexadecanal, decane, dodecane, tridecane, teradecane, pentadecane and longifolene, were not detected in the 2D GC-MS, however they were found in GC-MS/O. Therefore, the GC × GC-TOFMS combined with GC-MS/O could more comprehensively analyse the volatile profile in the stewed pork with different seasoning.

A large of multivariate data analysis techniques has been applied to metabolomics (Tromelin, Chabanet, Audouze, Koensgen, & Guichard, 2018; Wang, Fang, He, Dai, & Fang, 2016) and differentiation of meat products (Edwards et al., 2020; Reis, 2017; Zaid, 2019), including PCA, PLS-DA, HCA and PLSR in recent years. It was explained that the variation in the metabolic composition of chicken meat related to the ages by NMR spectroscopy coupled with PLS-DA method (Xiao, Ge, Zhou, Zhang, & Liao, 2019). The metabolite profiles of marinated meat with high pressure treatment were determined by NMR and PCA method, which demonstrated that high pressure treatment contributed to improve the palatable taste of marinated meat in soy sauce (Yang et al., 2018). Fourier transform infrared (FTIR) combined with PCA and HCA was a valuable tool to identify chicken or turkey meat adulteration in beef mixtures (Zaid, 2019). The visible-near infrared (VIS/NIR) spectroscopy and multivariated analysis for differentiation of normal and white striped turkey breasts (Zaid, Abu-Khalaf, Mudalal, & Petracci, 2020). These studies showed that multivariate statistical analysis was a powerful analytical technique to analyses the profile of low molecular weight compounds and discriminate meat products. However, only a few reports focused on the use of multivariate statistical analysis of volatile flavour compounds to discriminate meat of different breeds. (Pavlidis, Mallouchos, Ercolini, Panagou, & Nychas, 2019) has found that the 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. This reported has revealed that volatiles analysis and multiverse statistics analysis was a feasible method to distinguish of different varieties of meat. In this present study, the volatile compounds profiling and multiverse statistics analysis were a reliable method to characterize and differentiate boiled pork from different breeds. Additionally, considering the VIP scores and p-values (Mi, Shang, Jia, Zhang, & Fan, 2019), we have found that twelve dour-active compounds were determined as potential flavour markers for discrimination the different varieties of pork.

## *1.4. Relationship between flavour precursors and volatile compounds*

The research has revealed that the flavour precursors, such as small peptide, amino acids and nucleotides, were improved by enzymatic hydrolysis of meat proteins by flavour protease and neutral protease ([Kong et al., 2017](#_ENREF_29)). The free amino acids have an important contribution to the taste of pork, and plays a role in promoting taste during the maturation of pork ([Dashdorj, Amna, & Hwang, 2015](#_ENREF_12)). The high-temperature stewed pork with enzymatic degradation had the higher contents of UAAs, SAAs and BAAs than the traditional stewed pork. This could be due to the high temperature promoting the major release of FAA ([Diaz, Fernandez, De Fernando, de la Hoz, & Ordoñez, 1997](#_ENREF_13)). The Maillard reaction is a series of complex non-enzymatic reactions between carbonyl compounds and amino compounds, which is widely used in the preparation of meat flavours ([Dong et al., 2019](#_ENREF_14)). The thermal treatment is one of the most important factors affecting the reaction rate of the Maillard reaction ([Benzing-Purdie, Ripmeester, & Ratcliffe, 1985](#_ENREF_6)). The enzymatic hydrolysis and Maillard reaction could promote the generation of odour and taste compounds of processed food and the high temperature facilitates the Maillard reaction ([Liu, Liu, He, Song, & Chen, 2015](#_ENREF_35)). Through comparative analysis of volatile flavour compounds in stewed pork with different processing methods, it has been found that the volatile composition of high-temperature stewed pork (HS, HSE and HSEM) had a higher significantly level than that of traditional stewed pork (TS, TSE and TSEM). This result may be attributed to the fact that high temperature promotes Maillard reaction to produce more volatile compounds ([Benzing-Purdie et al., 1985](#_ENREF_6)). The lipids in pork is also an important flavour precursors ([Khan, Jo, & Tariq, 2015](#_ENREF_28)) and are closely related to the flavour formation of pork. The concentrations of SFA, MUFA, PUFA in high-temperature stewed pork decreased significantly (*P < 0.05*), when flavormyze, xylose, cysteine and thiamine was added. It may be due to the thermally induced reactions between fatty acid oxidation products, enzymatic hydrolysis products and Maillard reaction precursors could form a large number of volatile compounds.

## *1.5. Connection between the chapters of flavour research*

The volatile composition of stewed pork from four local brands were investigated using GC-MS/O, E-nose and sensory evaluation. In this way, we could fully understand the flavour of stewed pork to guide the processing of meat products. To the best of we knowledge, there are many factors that affect the flavour compounds of stewed pork, we have selected three main influencing factors (breeds of pork, spice recipes and processing methods) for research. The volatile compounds of boiled pork from three different breeds of pig were characterized and differentiated by GC-MS/O and E-nose combined with chemometrics analysis. The different spice recipes are usually used to affect the flavour of stewed pork. To study the influence of seasoning recipes on volatile profiles and sensory evaluation of stewed pork, the volatile compounds were investigated by GC-MS/O and GC × GC-TOFMS. During the processing of stewed pork, the flavor compounds are often lost and volatilized. In order to solve this problem, we discussed the influence of different processing methods on the flavour of stewed pork. The volatile and non-volatile compounds of stewed pork were determined by GC-MS/O, E-nose, HPLC and E-tongue.

# 2. General conclusion

The traditional stewed pork is a part of is representative Chinese meat products and is appreciated by consumers due to its distinct flavour characteristics. Flavour is one of the most important sensory attributes with regard to eating quality of stewed meat products (Qi, Liu, Zhou, & Xu, 2017). Most of the previous reports showed that the main factors affecting formation of flavour compounds of meat products were types of raw meat, spices, processing technology and storage conditions (Aaslyng & Meinert, 2017; Mancini, Paci, Dal Bosco, Mattioli, & Preziuso, 2019; Petričević, Radovčić, Lukić, Listeš, & Medić, 2018). Nowadays, many studies focused on the qualitative and quantitative analysis of the volatile compounds from the different meat products, few studies have been reported on the flavor profiling of stewed pork with different varieties, processing methods and spices. Moreover, in order to improve the desirable flavour in stewed pork, the flavour compounds of the traditional and high-temperature stewed pork were compared. The different stewed pork were prepared and analysed in this Ph.D. dissertation, including (1) four commercial stewed pork (DHM, DXC, HHT and TFH); (2) the boiled pork from three different breeds of pig (Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire)); (3) the fresh pork (FP) and stewed pork with different seasoning recipes (SP1, SP2, SP3, SP4, SP5 and SP6); (4) the traditional (TS, TSE and TSEM) and high-temperature (HS, HSE and TSEM).

## *2.1. Characterization of volatile compounds in Chinese stewed pork using SPME-GC-MS/O and E-nose*

The volatile composition of Chinese stewed pork from four brands (DHM, DXC, HHT and TFH) were investigated. A total of 62 volatile flavour compounds were detected in all pork samples, including 13 aldehydes, 9 alcohols, 5 ketone, 3 esters, 14 hydrocarbons, 2 ethers, 2 phenols, 7 furans, 3 N-containing and 4 S-containing compounds. Among them, the aldehydes and heterocyclic compounds play the key role in the stewed meat and meat broth. It was also found that the greatest number and highest levels of volatile compositions were detected in DXC and HHT, whereas TFH had the least number and lowest levels of volatile constituents. 24 odor-active compounds were defined, 7 of which were key odour-active compounds in stewed pork primarily contributing to the integral flavour of the stewed pork because of their higher OAVs. In order to better visualize the data of odor-active compounds and reduce the dimensions of the original variable, the PCA and PLS-DA were applied. This result showed the all stewed pork samples were clearly divided into three groups: DHM, HHT, and DXC-TFH. Considering the VIP scores and p-values, 9 odour-active compounds, heptanal, nonanal, 3-carene, d-limonene, β-phellandrene, p-cymene, eugenol, 2-ethylfuran and 2-pentylfuran, were determined as potential flavour markers for the discrimination of stewed pork. The intensities of fatty odour, meaty odour, roasted odour and soy sauce odour in DHM were higher than those in other samples, which could be mainly attributed to aldehydes and N- and S-containing compounds (e.g., nonanal, 2-nonenal isomer, 2,4-decadienal isomer, 2-acetylthiazole and 3-(methylthio)-propanol).

## *2.2. Characterization and discrimination of boiled pork from different breeds by volatiles profiling and chemomertrics analysis*

Regarding the volatile compounds of different breeds of boiled pork (DLY, TB and SMX) and different parts of the same breeds of boiled pork. It can be concluded that samples DLY were found to have higher proportion of aromatic compounds than two other samples (SMX and TB), the ratios of ethers, furans and S-containing compounds were the highest in sample SMX, and the most abundant compounds were aldehydes and ketones in samples TB. Which showed that the different kinds of pork have their own unique flavour composition. Statistical analysis showed that all odour-active compounds were significantly different (P < 0.05) in boiled pork from the three breeds. On the other hand, the OAVs of boiled pork of fore and hind leg muscles had not significant differences (P > 0.05). The response values of ten sensors of E-nose to boiled pork from fore and hind leg muscles had no significant differences, while the boiled pork of three pig breeds displayed the different response values. This result indicated that the influence of different pig breeds on flavour is greater than from different pig parts for boiled pork. Furthermore, it was a feasible method to differentiate the boiled pork from TB, SMX and DLY pigs by volatiles profiling and chemometrics analysis (PCA, AHC and PLS-DA).

## *2.3. Effect of seasoning addition on volatile composition and sensory properties of stewed pork*

In present study, we studied the changes and formation of flavour compounds in stewed pork with the addition of various spices. The volatile compounds in stewed pork from the different seasoning (stewing pork with water, salt, spices, soy sauce, sugar and cooking wine, SP1-SP6) were identified and quantified by GC-MS/O and GC × GC-TOFMS. The stewed pork with the addition of water and salt had the most abundant volatile compounds, especially aldehydes. This result indicated that the cooking pork with water and salt promoted lipid oxidation and amino acid degradation. According to the PCA of odour-active compounds, samples SP3, SP4, SP5 and SP6 were close each other in PC1-PC2, whereas samples SP3 was located on the opposite side of samples SP4, SP5 and SP6 in PC1-PC3. Which showed that the addition of spices had a significant influence on the flavour of stewed pork. The result of sensory evaluation had showed that the stronger spicy, caramel and soy sauce odour were presented in samples SP3, SP4, SP5 and SP6. This result was consistent with the PLSR analysis. The hexanal, 1-octen-3-ol and 2-pentylfuran were highly associated with meaty and fatty odour, while some aldehydes and (E)-2-ocetn-1-ol were strongly and negatively correlated with spicy, caramel and soy sauce note using PLSR.

## *2.4. Determination of volatile and non-volatile compounds of stewed pork from different processing methods*

The volatile and non-volatile compounds profile of stewed pork with the traditional (TS, TSE and TSEM) and high-temperature processing methods (HS, HSE and HSEM) were analysed. It was found that the high-temperature stewed pork (HS, HSE and HSEM) had a higher content of volatile composition than traditional stewed pork (TS, TSE and TSEM), especially sample HSEM. All stewed pork samples were clearly divided into two groups, including traditional stewed samples (TS, TSE and TSEM) and high-temperature stewed samples (HS, HSE and HSEM). Sample TS, TSE and TSEM had the lower contents of UAAs, SAAs and BAAs than sample HS, HSE and HSEM. The contents of 5’-nucleotides (AMP, GMP and IMP) in traditional stewed samples (TS, TSE and TSEM) showed higher level than that of high-temperature stewed pork (HS, HSE and HSEM). The contents of fatty acids in stewed pork samples decreased significantly (P < 0.05), when flavormyze, xylose, cysteine and thiamine was added. It can be concluded that high-temperature stewed pork (HS, HSE and HSEM) improve the taste and odour characteristic, of which high-temperature stewed pork with enzymatic hydrolysis and Maillard reaction was particularly prominent in the formation of odour compounds.

# 3. Perspective

Drawing on advanced theories and research results of food flavour analysis at home and abroad, the flavour components and odour-active compounds of four commercial stewed pork were qualitatively and quantitatively analysed using more advanced flavour compound analysis technology. Furthermore, each volatile compound was well evaluated its contribution to the overall flavour of stewed pork by OAVs and E-nose were also fast and simple analytical method to determine the whole flavour of stewed pork. These analyses enable us to better understand the aroma characteristics in Chinese stewed pork and monitor the changes of flavour quality of stewed pork. In addition, the volatiles profile of cooked pork from different varieties were analysed by GC-MS/O and E-nose. The results showed that the pig breeds are one of the key factors affecting the flavour of boiled pork, which is associated with the composition of SFA, MUFA and PUFA. The analytical technique, namely GC × GC-TOFMS combined with chemometrics, was successfully applied for the identification and quantitative analysis of volatile compounds in stewed pork with different seasoning recipes. The food seasoning was considered as natural flavour additives to improve the spicy flavour in stewed pork. Finally, to improve the flavour of stewed pork, the stewed pork of different processing methods was investigated. The high-temperature stewing with enzymatic hydrolysis and Maillard reaction impart the typical flavour to stewed pork and enhance more desirable flavour for consumers.

The flavour compounds in food were generally of various types, extremely small in content, and poor in stability (Aaslyng & Meinert, 2017). Therefore, the suitable separation technology has been needed for flavour compounds analysis, and the extraction method has greatly affected the reliability and accuracy of the analysis results. In 1990, the Pawliszyn research team of Waterloo University in Canada proposed SPME technology (Arthur & Pawliszyn, 1990), which has been experienced for 30 years so far. As a relatively mature pre-treatment technology, it has been widely used to extract volatile and semi-volatile compounds in food matrix (Lord & Pawliszyn, 2000; Risticevic et al., 2009; Stashenko & Martínez, 2007). Compared with the traditional extraction technology, this technology has integrated sampling, extraction, concentration, and injection. Which have the advantages of high sensitivity, low cost, less sample volume, good reproducibility and simple operation. The SPME method has been successfully used to extract the flavour components of some meat products and the research mainly has focused on pork products. García-González, Tena, Aparicio-Ruiz, & Morales (2008) have compared and analysed the flavour compounds of 41 Spanish and French hams from different origins and different maturity times and the results showed that the flavour active components are mainly alcohols, aldehydes and ketones. Chen, Su, He, Wu, & Shui (2019) have determined the differences in volatile compounds from pork meats of four different pig breeds using HS-SPME-GC-MS method. It was found that SDE is more suitable for analysing low volatile compounds, including fatty acids and esters, while HS-SPME is a more useful technique for analysing readily volatile components (Lin, Zhuang, Lei, Yang, & Zhao, 2013). A total of 203 volatile compounds were extracted from roast goat meat by DHS, SDE and SPME, of which DHS and SPME methods have good extraction efficiency for low-molecular-weight compounds, SDE method has extractsed more high-boiling volatile compounds. Compared with SDE and SPME, DHS method could extract many derivatives derived from Maillard reaction such as pyrazines, pyrrole and pyridine. However, it is recommended to use the simple and non-polluting SPME method when comparing a large number of samples or without a thermal desorption instrument (Madruga, Elmore, Dodson, & Mottram, 2009). For the reasons mentioned above, SPME method has been used to extract the flavour compounds of stewed pork in each chapter of this thesis. It has also been common to combine SPME with GC and MS technologies for flavour analysis. The comparison among preparation techniques is presented in Table 6 -1.

**Table 6 - 1:** comparison among preparation techniques commonly used in food flavour analysis.

|  |  |  |  |
| --- | --- | --- | --- |
| Preparation techniques | Advantages | Disadvantages | References |
| SDE | Low cost, simple equipment, solvent saving, high extraction efficiency, especially effective for extracting middle and high boiling compounds | The concentration process is easy to cause the loss of volatile components in the analyst, which has a greater impact on the heat-sensitive aroma components, leading to its decomposition and destruction | (Barra et al., 2007; Madruga et al., 2009; Prosen, Kokalj, Janeš, & Kreft, 2010) |
| SHS | Simple sample preparation, no other reagents | It is only suitable for the detection of highly volatile or high-content components. It is not possible to pre-concentrate the sample. It has poor precision and accuracy | (Marsili, 1999) |
| DHS or PT | It can effectively extract high and low boiling point volatile substances, prevent the decomposition or change of thermally unstable aroma substances at high temperature, the sample has few impurities, high sensitivity and low detection limit | Low efficiency and high cost when extracting low volatile components | (Barra et al., 2007; Mallia, Fernández-García, & Bosset, 2005) |
| SPME | It integrates sampling, extraction, concentration and sample injection, and requires less sample volume, reduces solvent consumption. It is fast and simple, non-polluting, and low cost, which is beneficial to extract low-boiling substances, maintain sample integrity, high sensitivity and precision degree | Loss of some water-soluble and high-boiling ingredients | (Risticevic et al., 2009; Stashenko & Martínez, 2007) |
| SPE | Low solvent consumption, good linearity, high recovery and sensitivity, and low detection limit | The use of organic solvents requires post-treatment processes such as separation and concentration. It takes a long time and is easy to lose low-boiling components | (Risticevic et al., 2009) |

Nowadays, the development of multi-dimensional chromatography has greatly improved the separation of complex organic compounds. GC × GC, as a powerful analytical technique for studying volatile components in complex matrices, could improve the resolution and sensitivity of the system. At the same time, it could be used in combination with mass spectrometry to accurately identify components. Adahchour et al. (2002) have detected volatile components of garlic using HS-SPME-GC × GC-FID, which was 10 to 50 times more sensitive than one-dimensional chromatography. The combination of comprehensive two-dimensional gas chromatography and selective detector could further enhance its practicality. Time-of-flight mass spectrometry is the most ideal detector for GC × GC (Duan et al., 2015). The combination of HS-SPME and GC×GC-TOF-MS has been used to analyse the flavour of food to classify and trace the origin of wine, honey, coffee and other foods. Adahchour, Wiewel, Verdel, Vreuls, & Udo (2005) successfully analysed volatile compounds of butter by HS-SPME-GC × GC-FID/TOF-MS method, and studied the effect of heat treatment on the composition of butter samples. The volatile components of honey from different regions and different plants were analysed using HS-SPMEGC × GC-TOF-MS, which proved that GC × GC-TOF-MS could be successfully used to identify the authenticity of honey (Stanimirova et al., 2010). Nowadays, GC × GC-TOF-MS has become more and more widely used in volatile compounds analysis in meat products, such as braised chicken (Duan et al., 2015), dry-cured ham (Wang et al., 2018) and grilled fish (Huang et al., 2019).

In order to identify the aroma active components in food, GC-MS/O and electronic sensory analysis technology have also been continuously developed in the research of flavour of food. Song & Liu (2018) reviewed the application of GC-MS/O method in meat products, soy sauce and chocolate, introducing some specific examples of the combination of SPME method and GC-MS/O. In addition, some researchers have used SPME in conjunction with electronic noses to identify and classify food varieties based on the "fingerprint" data (Ampuero, Bogdanov, & Bosset, 2004; Cimato et al., 2006; Stashenko & Martínez, 2007). In the current relevant reports, most researchers have analysed the flavour components of the product by GC-MS. Only a small part of volatile compounds with low content has contributed greatly to the product and could not be detected by GC-MS. Therefore, the contribution of flavour compounds to the overall flavour has not been determined using GC-MS. GC-O instrument was an effective method to combine GC separation ability with human olfaction, which select and evaluate odour from complex compounds (Plutowska & Wardencki, 2008). Therefore, GC-O and MS would be connected to play a greater role in the identification of volatile compounds and the judgment of the contribution to the whole flavour.

Aroma and taste are important indicators of meat quality and the sensory evaluation had been often used as a good evaluation method. However, the sensory evaluation has subjective factors, which would produce different results with factors such as the evaluator's physical condition and emotional changes. At the same time, the results of sensory evaluation were often vague and difficult to express with objective data. The rapid and low-cost evaluation method of meat quality has been widely concerned. The E-nose and E-tongue were composed of chemical sensors and a suitable pattern recognition system, which could obtain the comprehensive evaluation information of the sample from the response signal, that is, "fingerprint data"(Gutiérrez, Moreno-Barón, Pividori, Alegret, & del Valle, 2010; Lozano, Arroyo, Santos, Cabellos, & Horrillo, 2008). They could not only carry out simple comparative analysis of the aroma and taste information of different samples, but also establish a database by collecting standard sample information, and use chemometric methods to perform qualitative and quantitative analysis of unknown samples. The comparison among preparation techniques is presented in Table 6 -2.

**Table 6 - 2:** Characteristics of different analytical methods used in detection of flavour compounds.

|  |  |  |  |
| --- | --- | --- | --- |
| Analytical methods | Advantages | Disadvantages | References |
| GC-MS | Accurately quantify and characterize the flavour compounds and high detection sensitivity | It is unable to determine the contribution of a single odour-active compound to the overall flavour and identify low-level flavour compounds | (Petričević et al., 2018) |
| GC-O | It can identify key flavour compounds in the product and has high detection sensitivity | It cannot be used independently, and needs to be used with GC-MS. The price of the instrument is higher | (Huang et al., 2019) |
| GC-MS/O | It is particularly favourable and efficient for the identification or picking-up of aroma-active compounds from numerous volatile constituents | The price of the instrument is higher and the test results are greatly affected by the environment | (H. Liu et al., 2019; H. Song & Liu, 2018) |
| GC × GC-TOFMS | It offers higher separation power and the two-dimensional elution pattern is meaningful and facilitate analyst identification and sample fingerprinting | The artificial analysis of data is easy to cause errors and the qualitative analysis of flavour compounds is complicated. The price of the instrument is higher | (Duan et al., 2015b; W. Wang et al., 2018) |
| E-nose | It is a rapid, easy, reliable, accurate and non-destructive analysis technique | It cannot accurately analyze the characteristics of each compound | (Zhou, Chong, Ding, Gu, & Liu, 2016) |
| E-tongue | It is a rapid, easy, reliable low-cost evaluation method | It cannot accurately describe the source of different tastes | (Haddi et al., 2014) |

In order to conduct a more in-depth study of the flavour compounds in the stewed pork, some suggestions were put forward: (1) to further confirm the contribution of the detected main aroma compounds to the overall flavour. More accurate quantitative analysis methods should be adopted to achieve more accurate results, such as stable isotop dilution analysis (SIDA) to reduce experimental errors. (2) Due to limited time, it is recommended that the model system be used to investigate the formation of aroma active compounds during the processing of stewed pork in future experiments. (3) The identified odour-active compounds in the stewed pork should be subjected to further the verification experiments of flavour compounds, such as flavour recombination and omission test. (4) In fact, during the processing of stewed pork, a small amount of cooking off-odour compounds are often accompanied, how to eliminate these off-odour compounds can be further studied. (5) This study mainly focuses on the volatile compounds of stewed pork, and there were a few studies on non-volatile compounds, which can be strengthened. (6) The volatiles fingerprinting of stewed pork could be establish due to a large of amount of flavour compounds data. (7) The mix spice was a mixture of 18 kinds of spices in the thesis and each spice has its own unique properties. We could select the spices that are commonly used in the stewed pork for systematic flavour analysis. The characteristic volatile compound was extracted from the spice, and then interact with myofibrillar proteins to expose the flavour binding mechanism.

# 4. References

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Appendix - publications

**1. Articles**

(1) **Han, D.**, Mi, S., Zhang, C. H., Li, J., Song, H. L., Fauconnier, M. L., & Tyteca, E. (2019). Characterization and Discrimination of Chinese Marinated Pork Hocks by Volatile Compound Profiling Using Solid Phase Microextraction Gas Chromatography-Mass Spectrometry/Olfactometry, Electronic Nose and Chemometrics. *Molecules*, *24*(7), 1385. (Published)

(2) **Han, D.**, Zhang, C. H., Fauconnier, M. L., & Mi, S. (2020). Characterization and differentiation of boiled pork from Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire) pigs by volatiles profiling and chemometrics analysis. *Food Research International*, *130*, 108910. (Published)

(3) **Han, D.**, Wang, H., Zhang, C. H., & Fauconnier, M. L. Effect of seasoning addition on volatile composition and sensory properties of stewed pork. *Food Chemistry*. (Submitted)

(4) **Han, D.**, Wang, H., Zhang, C. H., & Fauconnier, M. L. A combination enzymatic hydrolysis and Maillard reaction to enhance volatile flavor in quantitative stewed pork. *International Journal of Food Science & Technology*. (Submitted)

**2. Patents**

(1) Zhang, C. H., **Han, D.**, & Li, Xia. Analysis method of flavour active compounds in stewed meat products. 201610137308.X.

(2) Zhang, C. H., **Han, D.**, & Li, Xia. Identification method of key flavour active compounds in stewed meat products. CN17B8914A.

(3) Zhang, C. H., **Han, D.**, & Li, Xia. Analysis method of taste compounds profile in meat product. CN19B11527A.

(4) Zhang, C. H., **Han, D.**, & Li, Xia. Identification method of Tibetan pig and its meat products based on chemometric analysis. CN19B11784A.

**3. Flash presentations and posters**

(1) 2017 China Animal Products Processing Technology Conference: **Poster Presentation** of own project, **1st author** (Nanjing) entitled “Profile analysis of odor active compounds in stewed pork”. 01/11/2017-01/11/2017.

(2) 25th National Symposium for Applied Biological Sciences (NSABS): **Poster Presentation** of own project, **1st author** (Gembloux) entitled “Characterization and differentiation of boiled pork from Tibetan, Sanmenxia and Duroc × (Landrace ×Yorkshire) pigs by volatiles profiling and chemomerics analysis” and **the author** was invited to present a flash presentation. 31/01/2020.