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Agro-Bio Tech

UNTARGETED AND TARGETED AROMA PROFILE ANALYSIS OF RAW AND BAKED JUJUBE

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Year: 2022

COMMUNAUTÉ FRANÇAISE DE BELGIQUE

UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

UNTARGETED AND TARGETED AROMA PROFILE ANALYSIS OF RAW AND BAKED JUJUBE

Analyse Non Ciblée et Ciblée du Profil Aromatique des Jujubes Rouges Crus et Cuits

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Année civile: 2022

Résumé

Yening QIAO (2022). 'Analyse non ciblée et ciblée du profil aromatique des jujubes rouges crus et cuits' (Thèse de doctorat en anglais). Gembloux, Belgique, Gembloux Agro-Bio Tech, Université de Liège, 194 pages, 24 tableaux, 48 figures.

Résumé :

Le jujube d'hiver est décrit comme ayant des caractéristiques aromatiques fraîches et vertes. Cependant, il n'y a pas eu de recherche systématique pour clarifier la répartition géographique des profils volatils des jujubes d'hiver. La qualité du jujube rouge de la province du Xinjiang, en Chine, est supérieure à celle des autres régions, mais aucune étude n'a été menée pour révéler ses caractéristiques aromatiques. De plus, l'arôme du jujube rouge cuit au four n'a pas encore été révélé.

Premièrement, la chromatographie en phase gazeuse-spectrométrie de mobilité ionique (GC-IMS), la spectrométrie de masse GC (MS) et le nez E ont été appliqués pour l'analyse de l'arôme du jujube d'hiver de différentes régions. Les résultats ont montré que l'alcool benzylique, l'acide octanoïque, le 2-hexénal, le linalol, le 2-nonénal et le décanoate d'éthyle étaient les composés les plus courants présents dans tous les jujubes d'hiver. Le 1-pentène-3-ol, l'hexanoate d'éthyle, le laurate de méthyle et le 2-méthylbenzaldéhyde ont été induits comme marqueurs potentiels de XJAKS avec un arôme vert et fruité, SXYC pourrait être marqué par l'acétone et le 2-méthoxyphénol avec un arôme boisé et piquant.

Deuxièmement, six cultivars de jujubes rouges (cv. JC, JZ, HZ, QYX, HTDZ et YZ) cultivés dans la province du Xinjiang, en Chine, ont été collectés. GC-IMS et E-Nose ont été utilisés pour étudier le profil volatil. JC, JZ, HZ et YZ étaient différents des autres, tandis que QYX et HTDZ étaient similaires. L'acétoïne, l'E-2-hexanol, l'hexanal, l'acide acétique et l'acétate d'éthyle étaient cruciaux pour la classification. L'analyse de corrélation a montré que le jujube ID pourrait être lié au phénylacétaldéhyde et à l'acide isobutanoïque formés par la transamination ou la déshydrogénation des acides aminés, tandis que l'attribut sucré était corrélé avec les acides aminés, notamment la thréonine, l'acide glutamique, la glycine, l'alanine, etc.

Troisièmement, l'arôme particulier du jujube rouge cuit au four n'a pas été évalué jusqu'à présent. Les sciences sensorielles moléculaires ont été appliquées pour l'identification des odeurs clés. Le 5-méthyl-2-furancarboxaldéhyde, la β-damascénone, le benzaldéhyde, le limonène, l'acide hexanoïque, la 5-butyltétrahydro-2-furanone et le DDMP étaient les principaux odorants du jujube rouge cuit. Le processus de cuisson a stimulé la formation de composés d'hydrocarbures aromatiques comme le 1H-pyrrole-2-carboxaldéhyde et le DDMP.

Enfin, HS-SPME-GC-MS/MS a été appliqué pour analyser quantitativement les composés ciblés. Le DDMP (2,3-dihydro-3,5-dihydroxy-6-méthyl-4H-pyran-

4-one), formé lors de la cuisson du jujube rouge, se caractérise par une odeur amère et une toxicité potentielle. Les pyrazines alkyl-substituées perçues comme des odeurs de noisette, de rôti ou sucrées contribuent beaucoup à l'arôme de jujube rouge. La courbe standard du DDMP était linéaire entre 0,01 et 100 µg/mL, avec une limite de détection à 0,1 ng/g sous le seuil sensoriel du DDMP (2,06 µg/g). De bons coefficients de corrélation linéaire (0,9947 à 0,9988) ont été obtenus sur les plages de 1 à 10 000 ng/mL pour les alkylpyrazines. Les limites de détection (LOD) et les limites de quantification (LOQ) des pyrazines se situaient entre 0,5 et 10 pg/g et 1,5 et 30 pg/g, respectivement. La triméthylpyrazine, qui représentait 38,71% à 59,50% de la pyrazine totale, était la principale alkylpyrazine dans le jujube rouge brut. La méthylpyrazine représentait 35,52% à 49,76%, la proportion la plus élevée d'alkyl pyrazine dans le jujube rouge cuit. Le processus de cuisson pourrait stimuler la désalkylation des alkylpyrazines.

Mots clés: jujube d'hiver, jujube rouge, analyse non ciblée, analyse ciblée, HS-GC-IMS, HS-SPME-GC/MS-O, HS-SPME-GC-MS/MS

Abstract

Yening QIAO (2022). 'Untargeted and targeted aroma profile analysis of raw and baked jujube' (PhD Dissertation in English).

Gembloux, Belgique, Gembloux Agro-Bio Tech, Universite de Liège, 194 pages, 24 tables, 48 figures.

Summary:

Winter jujube is described as having fresh and green aroma characteristics. However, there was no systematic research to clarify the geographical distribution effect on the aroma of winter jujube. The quality of red jujube from Xinjiang Province, China, is superior to other regions, but no studies have been carried out to reveal its regional aroma characteristics. Besides, the aroma of baked red jujube has not been revealed yet.

Firstly, gas chromatography-ion mobility spectrometry (GC-IMS), GC-mass spectrometry (MS), and E-nose were applied for the aroma analysis of winter jujube from different regions. The results showed benzyl alcohol, octanoic acid, 2-hexenal, linalool, 2-nonenal, and ethyl decanoate were the most common compounds presented in all winter jujubes. 1-Penten-3-ol, ethyl hexanoate, methyl laurate, and 2 -methylbenzaldehyde were induced as the potential markers of XJAKS with a green and fruity aroma. SXYC could be labeled by acetone and 2-methoxyphenol with woody and pungent aroma.

Secondly, six cultivars of red jujubes (cv. JC, JZ, HZ, QYX, HTDZ, and YZ) grown in Xinjiang Province, China were collected. GC-IMS and E-Nose were used to study the volatile profile. JC, JZ, HZ, and YZ were different from the others, while QYX and HTDZ were similar to each other. Acetoin, E-2-hexanol, hexanal, acetic acid, and ethyl acetate were crucial for the classification. Correlation analysis showed jujube ID might be related to phenylacetaldehyde and isobutanoic acid that formed by the transamination or dehydrogenation of amino acids.Meanwhile, the sweet attribute was correlated with amino acids including threonine, glutamic acid, etc.

Thirdly, the particular aroma of baked red jujube has not been evaluated until now. Molecular sensory sciences were applied for the identification of key odors. 5-Methyl-2-furancarboxaldehyde, β-damascenone, benzaldehyde, limonene, hexanoic acid, 5-butyltetrahydro-2-furanone, and DDMP were the key odorants for baked red jujube. The baking process stimulated the formation of aromatic hydrocarbon compounds like 1H-pyrrole-2-carboxaldehyde and DDMP.

Finally, HS-SPME-GC-MS/MS was applied to quantitatively analyze targeted compounds. DDMP (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one), formed during the baking of red jujube, is characterized by bitter odor and potential toxicity. Alkyl-substituted pyrazines perceived as nutty, roasted, or sweet odors contribute a lot to red jujube aroma. The standard curve of DDMP was linear between the ranges of 0.01 and 100 μg/mL, with a limit of detection at 0.1 ng/g below the sensory threshold of DDMP (2.06 μg/g). Good linear correlation coefficients (0.9947 – 0.9988) were obtained over the ranges of 1 – 10000 ng/mL for the alkyl pyrazines. The limits of detection (LODs) and limits of quantitation (LOQs) for the pyrazines were in the range of 0.5–10 pg/g and 1.5–30 pg/g, respectively. Trimethylpyrazine, which accounted for 38.71% – 59.50% of the total pyrazine, was the main alkyl pyrazine in raw red jujube. Methylpyrazine accounted for 35.52% – 49.76%, the highest portion of alkyl pyrazine in baked red jujube. The baking process might stimulate the dealkylation of alkyl pyrazines.

Keywords: winter jujube, red jujube, untargeted analysis, targeted analysis, HS-GC-IMS, HS-SPME-GC/MS-O, HS-SPME-GC-MS/MS

Acknowledgments

First and foremost, I would like to extend my deeply sincere gratitude to all of the parties who helped and supported my P.h.D dissertation.

I would like to extend my sincere gratitude to my distinguished and respectable supervisor, Professor Giorgia Purcaro, who gives me a lot of inspiration in the research, and guides me to write this thesis. Her profound professional knowledge, professionalism, and strict self-discipline inspire me to continuously improve the thesis, to improve myself, and to have a positive attitude towards life.

I feel so lucky to be a member of this team. We progress together, help each other, and grow together. The experience in the Analytical Chemistry laboratory, University of Liège, Belgium, is priceless for me.

There are also a lot of thanks to my co-supervisor Professor Jinfeng Bi, who always supports me with my study, encourages me to enrich my professional knowledge and improve my social practice ability. Under his guidance, I insist on my P.h.D study and life. I am constantly encouraged to improve myself, strictly discipline myself, overcome difficulties, improve my comprehensive ability, and maintain continuous progress.

Secondly, I also want to express my sincere gratitude to Associate Professor Qinqin Chen, who gives me detailed guidance and suggestions in my project, carefully and patiently revising the papers and dissertation. Her serious, responsible, courageous, persistent, optimistic and positive attitude towards work and life will affect my subsequent work and life. I feel very lucky to study with her guidance and company.

Besides, I will also express my sincere gratitude to other professors and teachers who has given me constructive suggestion to my research and thesis, and thanks for the guidance in the aroma profile analysis in the past four years.

Life is real, life is earnest. There have been many things in my life that seem to be no different from other people's lives. People come and go, and I struggle to defeat the psychological giant. In this process, I lost my confidence countless times and regained hope. My progress is inseparable from the support of my families and friends in China and Belgium.

I must say thanks to associate researchers, Xuan Liu, Qinqin Chen, Jianyong Yi, Xin Jin, and assistant researches, Jian Lv, Xinye Wu, Mo Zhou, and Xuan Li. They have given me great guidance and help in study and life. I would like to say thanks to my friends in Analytical Chemistry Laboratory, Steven Mascrez, Damien Eggermont, Gregory Bauwens, Angelica Fina, Alex Glineur, and Veronika Křešťáková. I feel so warm and loved during the days here in Gembloux-Biotech.

Besides, I also have to thank all members of Laboratory of Fruit and Vegetable Processing of Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, provided me with a friendly and active learning and living environment. They are Jian Peng, Jianxin Song, Xiao Li, Chongting Guo, Lina Hu, Dazhi Liu, Yuanyuan Zhao, Jianing Liu, Ying Lv, and so on. Thanks to these students for their encouragement, support and company, I have the confidence to keep moving forward in my studies.

Last but not least, I want to express my sincere gratitude and love to my parents, as well as my brother and sister who support me, care for me, and love me. I am so lucky to have your unconditional and all-time material and spiritual support on the road of life. It is nice to have you, even if there may be more ups and downs in my life, I still have the courage to keep going.

Yening Qiao

15/03/2022 in Gembloux, Belgium

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List of Abbreviations

ALA, Alanine
ARG, Arginine
ASP, Aspartic acid
CAR, Carboxen
CIT, Citrulline
CP, Conducting polymers
DDMP, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one
DT, Drift time
DVB, Divinylbenzene
DVB/CAR/PDMS, Divinylbenzene/carbonWR/polydimethylsiloxane
E-nose, Electronic nose
FD, Flavor dilution
FID, Flame ionization detector
O, Olfactometry
GLU, Glutamic acid
GLY, Glycine
HBHH, Hebei Province, Huanghua City
HIS, Histidine
HPAEC-PAD, High-performance anion-exchange chromatography equipped with pulsed amperometric detection
HS-GC-IMS, Head space gas chromatography ion mobility spectrometry
HS-SPME/GC-MS, Head space solid phase gas chromatography mass spectrometry
HTDZ, Hetiandazao
HZ, Huizao
ID, Identity
ILE, Isoleucine
IS, Internal standard
JC, Jinchang
JZ, Junzao
LAV, Laboratory analytical viewer
LEU, Leucine
LOD, Limit of detection
LOQ, Limit of quantitation

LYS, Lysine
MET, Methionine
MOS, Metal-oxidesemiconductor
MRM, Multiple reaction monitoring
MS/MS, Tandem mass spectrometry
OAV, Odor activity values
PA, Polyacrylate
PCA, Principal component analysis
PDMS, Polydimethylsiloxane
PDMS/DVB, Polydimethylsiloxane/divinylbenzene
PLS-DA, Partial least squares discriminant analysis
PRO, Proline
QCM, Quartz crystal microbalance
QDA, Quantitative descriptive analysis
QDA, Quantitative descriptive analysis
QYX, Qiyuexian
RDA, Redundancy analysis
RI, Retention index
RIP, Reaction ion peak
RSD, Relative standard deviation
RSD, Relative standard deviation
S/N, Signal-to-noise
SAW, Surface acoustic wave
SDYT, Shandong Province, Yantai City
SDZH, Shandong Province, Zhanhua City
SER, Serine
SNXDL, Shannxi Province, Dali City
SXLY, Shanxi Province, Linyi City
SXYC, Shanxi Province, Yuncheng City
THR, Threonine
TYR, Tyrosine
VAL, Valine
VIP, Variable influence on projection
VOCs, Volatile organic compounds
XJAKS, Xinjiang Uygur Autonomous Region, Akesu

XJKEL, Xinjiang Uygur Autonomous Region, Kuerle City
YZ, Yuanzao

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Chapter Ⅰ. General Introduction

1.Background

Jujube (*Ziziphus jujuba* Mill.) is consumed as dry fruit, herbal medicine, as well as ingredients for traditional Chinese dishes. China is the largest jujube producer, contributing over 90% of the world jujube production and the only country exporting jujube fruits (Wang et al., 2016).

Jujube is generally considered a nutritious fruit as it contains biological compounds including triterpenic acids, flavonoids, cerebrosides, amino acids, phenolic acids, mineral constituents, and polysaccharides (Guo et al., 2009; Pawlowska et al., 2009; Choi et al., 2011; Gao et al., 2011; Zhao et al., 2008.). Usually, jujube is processed into many products in the forms of paste, puree, syrup, confection, cake, tea, wine, vinegar, etc. by ways of fermentation, baking, and heating, etc. In addition, jujube is also used to make flavorings and fragrances for its special and pleasant aroma (Li et al., 2007).

There are already some studies on the aroma of jujube since 2014 (Bi et al., 2014). The volatile organic components of table jujube (cultivars of Muzao, Yuanzao, Lizao, Xiangzao, Banzao, Dongzao, Jinsixiaozao, Pingdingdazao, Lingwuchangzao, Junzao, Banzao, Dabailing, Jinsixiaozao, Hupingzao, Lingbaozao, Yuanlingzao, Jidanzao, Lizao, Baodeyouzao, Jinzao, Pingshunjunzao, Xiangzao, Pozao, Neihuangbianhesuan, and Huizao, Hamidazao) from the regions of Shanxi Province, Shandong Province, Hebei Province, Liaoning Province, Ningxia Province, Xinjiang Province, and Hunan Province in China (Chen et al., 2015; Wang et al., 2019; Liu et al., 2021; Song et al., 2019; Yang et al., 2019). Besides, the aroma characteristics of dried jujube cultivars of Tangzao, Muzao, Lizao, Qingrunhongzao, Jinsixiaozao, Youzao, and Yuzao from the places of Hebei Province, Shaanxi Province, and Xinjiang Province in China, and the province of Alicante, Spain have also been clarified (Wang et al., 2018; Zhu&Xiao, 2018; Wojdyło et al., 2016). Furthermore, some researches were also carried out on the processed jujube's aroma profile, including dried jujube and jujube wine (Wang et al., 2016; Song et al., 2020; Bi et al., 2014; Xia et al., 2020; Zhao et al., 2021).

Recent studies showed the aroma profile of fresh jujube was mostly composed of aldehydes (Chen et al., 2018; Song et al., 2019; Yang et al., 2019). In addition, the compounds of (E)-2-hexenal, (E)-2-heptenal, (E)-2-octenal, hexanal, 2,3-butanedione, β-damascenone, ethyl hexanoate, 3-mercaptohexyl acetate, and 2,5-dimethylpyrazine were the key odor active compounds of the red jujubes (cv. Tangzao; Muzao; Lizao; Qingrunhongzao; Jinsixiaozao; Youzao; and Yuzao from the production areas of Hebei, Shaanxi, and Xinjiang Province, China) (Zhu & Xiao, 2018). In terms of the processing effect on jujube aroma, the fermentation step would contribute to the formation of the total volatile compounds as well as alcohols, esters, acids, and aldehydes (Cai et al., 2020).

The techniques of aroma analysis were mainly E-nose, HS-SPME/GC-MS, HS-GC-IMS, and molecular sensory sciences (GC-MS, GC-MS-O, sensory evaluation, recombination test, and omission test). Among these techniques, E-nose has been applied successfully in many practices like the adulteration, classification, and authentication of food (Mildner-Szkudlarz & Jelen, 2010; Rodríguez et al., 2014;

Kovacs et al., 2010). HS-SPME/GC-MS showed its advantage of sensitivity, precision, and non-invasive and undemanding sampling in many areas (Feizi, 2021). Besides, for the targeted compounds' analysis in food and environment areas, HS-SPME-GC-MS/MS was more widely applied (Hopfer et al., 2016).

2.Objective

The objective of the study is to define the aroma quality fingerprints of fresh jujube (cv. winter jujube) from different regions, cultivars of dried jujube from Xinjiang Province, China, and processed dried jujube (cv. Junzao, Xinjiang Province, China) to better characterize their unique qualities, and to protect the specialty, also to reveal the relationship between sensory quality, targeted compounds and chemical composition, as well as some transformation processes.

Four specific objectives were pursued during this work and are summarized as follows:

(1) to investigate the volatile profiles of winter jujubes from different regions and identify the potential markers of winter jujube from each specific region.

(2) to discriminate the aroma profile of red jujube cultivars cultivated in Xinjiang Province and reveal the relationship between the sensory perception and the characteristic VOCs and metabolite precursors.

(3) to reveal the key odor active compounds' changes in the baking process of red jujube (cv.Junzao).

(4) to develop a sensitive, accurate, easy to operate, and solvent - free method to track the changes of certain compounds in red jujube.

3.Research outline

The literature review introduces the work undertaken in this thesis by describing the research status of raw and processed jujube aroma, the research progress of the techniques that can be used for the analysis of aroma, together with the introduction of the statistic methods used in this project (Chapter II).

The research content of the thesis work is shown in Figure 1-1. Firstly, The composition of VOCs of winter jujubes from eight different regions in China was identified. The specific VOCs that played an important role in the discrimination was highlighted. Meanwhile, the correlation analysis between the differential VOCs (VIP scores ≥ 1) and E-nose sensors was performed to indicate the potential markers for the winter jujubes (Chapter III).

Secondly, the effect of cultivar on red jujube aroma characteristics from Xinjiang Province, China, was investigated. In addition, the chemical composition of the non-volatile metabolites, i.e. fatty acids, amino acids, organic acids, and sugars, along with sensory attributes were determined. At last, the correlation analysis between sensory quality and chemical composition was carried out (Chapter IV).

Thirdly, the influence of baking on the key odor active compounds changes of red jujube (cv. Junzao) was studied. Moreover, the changes in sensory quality was evaluated as well (Chapter V).

Fourthly, the changes in heterocyclic compounds were further monitored with the application of targeted analytical methods(Chapter VI&VII).

Overall, this study allows a better understanding of the volatile profile of table jujube , red jujube, and processed red jujube through the application of instrumental analytical methods, statistical methods, and molecular sensory sciences on volatile profile identification of table jujube, red jujube, and processed red jujube. The geographical and cultivar differentiation of jujube aroma is accomplished. After that, the changes of key odor active compounds in the baking process are investigated. At last, a developed method based on HS-SPME-GC-MS/MS is applied to track the effect of baking on certain volatile compounds.

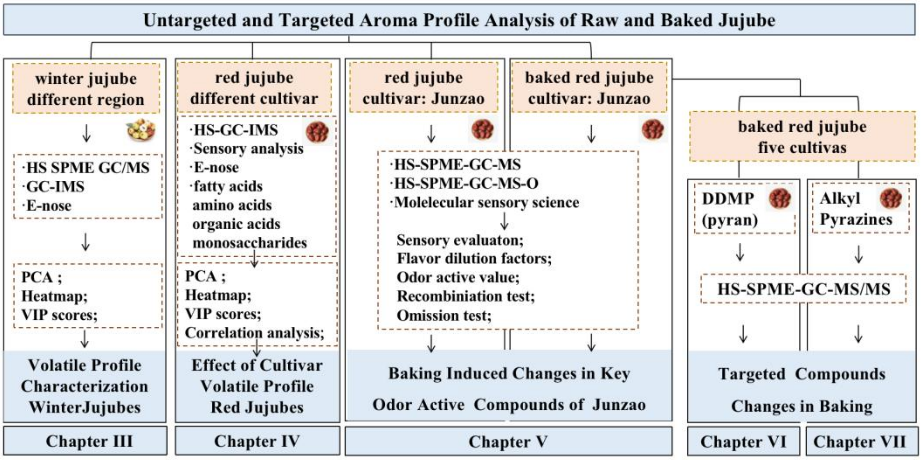

Figure 1-1 Technical route of the research content

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Chapter Ⅱ. Literature review on winter jujube and red jujube, techniques of aroma analysis, and research progress of jujube aroma

Abstract

This review covers recent research advances in techniques of aroma analysis, research progress of jujube aroma, as well as the application of statistic methods. Jujube is a homology of food and medicine with a special and attractive aroma. There are various cultivars and wide distributed planting places for fresh jujube and red jujube in the origin place, China. Meanwhile, it lacks comprehensive studies on the effect of cultivar and geographical distribution on aroma profile. Furthermore, the study on the aroma of the representative fresh jujube, winter jujube, from different regions has not been studied yet. In addition, the particular aroma profile of red jujube cultivated in the biggest production area, i.e., Xinjiang Province, together with the correlation analysis between sensory characteristics and chemical composition, remained to be investigated. This review focuses on the comprehensive introduction of jujube, aroma analysis techniques of fruit, and the research progress on the aroma composition of raw and processed jujube.

Keywords: Winter jujube, Red jujube, Techniques, Aroma analysis

1. Introduction

1.1. Winter jujube and red jujube

Jujube (*Ziziphus jujuba* Mill.), also called Chinese dates, belonging to the botanical *Rhamnaceae* family, was originated from China and has been cultivated in China for over 4000 years (Liu et al., 2016). Jujubes were reported to exhibit a series of bioactivities including antiadipogenic, antidiabetic, antiepileptic, anti-inflammatory, and antimicrobial, etc. (Kubota et al., 2009; Michel et al., 2011; Pahuja et al.; 2011; Yu et al.; 2012; Daneshmand et al.; 2013). Thus jujube has been consumed as a homology of food and medicine in daily life for a long time. The data from the Chinese National Bureau of Statics showed that 3.31 million of hectares are cultivated with jujube over China, with an output of 7.36 million tons (2018). The jujube production of China occupies 98% of the world, with an annual export of 14,600 tons, ranking the largest supplier of jujubes (2018). The geographical distribution of jujube is 19–44°N, 76–124°E, which includes Xinjiang, Shanxi, Shaanxi, Hebei, Henan, Shandong, Gansu, Liaoning, and Ningxia provinces of China (Figure 2-1). After a long period of travel, it is now grown in many places around the world (Table 2-1).

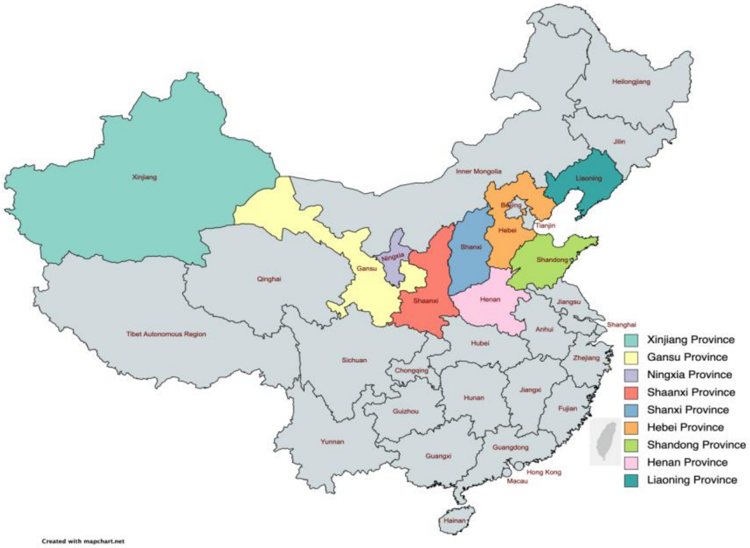


Figure 2-1 Geographical distribution of jujube planting in China

Table 2-1 Geographical distribution of jujube planting worldwide

continent	country
Asia	Afghanistan, Armenia, Azerbaijan, Bangladesh, Myanmar, China, Cyprus, India, Iraq, Israel, Japan, Kyrgyzstan, Lebanon, Malaysia, Mongolia, North Korea, Pakistan, Palestine, South Korea, Syria, Thailand, Turkey, Turkmenistan, Uzbekistan
Europe	Bulgaria, England, France, Germany, Greece, Italy, Czech Republic, Macedonia, Portugal, Romania, Russia, Serbia, Slovenia, Spain, Ukraine
Africa	Egypt, Tanzania, Tunisia
North America	Canada, the United States
Oceania	Australia, New Zealand

There are four development stages for jujube fruit, namely white mature (light green), crisp mature (white-red), fully mature (red), and fully ripe (dehydrated brown) (Figure 2-2) (Liu et al., 2016). As of now, there are over 1000 cultivars of jujube around China that could be classified into table (4%), dried/dehydrated (80%), candied (5%), multipurpose (10%), and ornamental group (1%) based on their primary usage (Liu et al., 2016). Usually, there are two kinds of jujubes sold in the markets: fresh mature jujubes eaten as fruits (table fruit), and dried mature jujubes (dried fruit) used as fruits or medicinal herbs (Figure 2-2). Dried jujube fruits are usually prepared by the sun-drying and post-drying (hot air drying, heat-pump drying, etc.) steps to extend the shelf life.

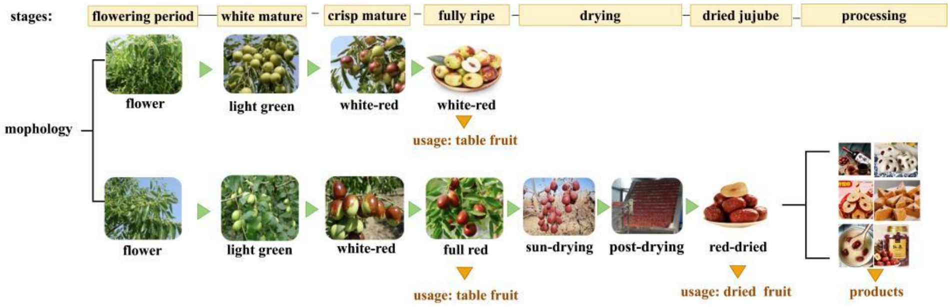


Figure 2-2 The mature stages of jujube

For table jujube, there are cultivars of winter jujube, Zaocuiwang, Chaoyangdazao, etc., of which winter jujube is a representative variety of fresh jujube, widely loved by consumers for its delicious taste and a pleasant aroma.

For dried jujube, also called red jujube, there are cultivars of Junzao, Huizao, Jinsixiaozao, Lingwuchnagzao, Mingshanzazao, Jinchang, Muzao, Yuanzao, Linzexiaozao, Hupingzao, Fupingzao, Banzao, etc. In terms of the distribution of the producing areas for red jujube, Xinjiang province is the largest production region in China with an annual output of more than 1.45 million tons. What's more, the quality of red jujubes from Xinjiang province is superior to those from other regions because of the area's low rainfall with periodic drought, abundant sunshine, and substantial differences between day and night temperatures (Wang et al., 2019).

When referring to the processing techniques, jujube fruit could be made into jujube tea, cake, bread, wine, and also candied snacks (jams, jelly, and pickles) through the techniques of baking, steaming, fermentation, and candied treatment (Rashwan et al., 2020).

1.2. Techniques of aroma analysis

Aroma is one of the most important points of food sensory quality, consumer acceptability, and driven force in purchase. Volatile chemicals comprise only a small portion (0.01% to 0.001%) of the fruit composition but gives a significant contribution to the overall aroma (Buttery, 1981). Therefore, the analysis of volatile chemicals provides the knowledge needed to objectify food aroma quality and enables the improvement of the understanding on the aroma attributes correlating their formation with their chemical precursors.

There are different kinds of instrumental methods to separate and characterize the aroma compounds. Direct extraction can be performed, such as steam distillation extraction, but usually headspace extraction is preferred, either static, dynamic or using the purge and trap approach (Marsili, 1996). The headspace (HS) technique will be applied in this project as it has the advantages of simple sample preparation, no need of any reagents, and relatively low cost (Marsili, 1996). Secondly, electronic nose (E-nose) is applied to obtain the overall volatile composition of the samples (Vera et al., 2011). Thirdly, gas chromatography, mass spectrometry, together with olfactrometry technique were involved for the identification of the volatiles. Besides, sensory sciences is also an important way to better understand the aroma attributes, differentiate the volatiles that has crucial impact, and assess consumer preferences. In this part, the instrumental and sensory aroma analysis methods that can be used for the untargeted and targeted analysis of jujube aroma will be introduced in detail.

1.2.1. Molecular sensory sciences

The concept of molecular sensory sciences was first introduced by Peter Schieberle in 2007, as the science aiming to identify the key odor active compounds based on instruments (gas chromatography mass-spectrometry (GC-MS) and Olfactometry (O)) and sensory sciences (sensory evaluation, recombination test, and omission test) (Steinhaus & Schieberle, 2007). Among the mentioned instrumental methods, GC-MS was applied for the qualification and quantitation of the volatiles, which could be used for the calculation of odor activity values (OAV) and the reference concentration for recombination and omission test. GC-MS-O was an efficient instrument for the identification of volatiles through retention indexes and odor descriptions (Figure 2-3) (Song & Liu, 2018). To determine the potency of odorants that contribute most to an aroma characteristic, flavor dilution (FD) factors were applied in the GC-MS-O procedure (Grosch, 1993). And the FD factor can be determined in either of the following three ways: I) dilution by varying the sample volume; II) dilution by diluting the sample; III) dilution through adjusting the GC injector split ratio (Feng et al., 2015).

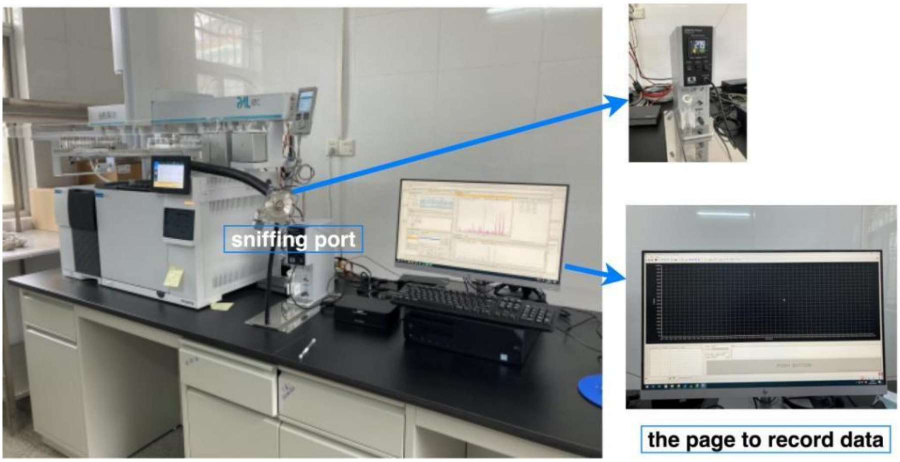


Figure 2-3 The system of GC-MS-O

The science of sensory evaluation developed rapidly since the 1950s (Lawless et al., 2010). Sensory evaluation can give a measurement of the aroma characteristics of the samples through quantitative descriptive analysis (QDA), which is performed by a group of trained panelists (Saldaña et al., 2021). OAV is the ratio of concentration to the threshold of a certain compound, which can reflect its contribution to a specific aroma (Steinhaus & Schieberle, 2007). In detail, the compounds with OAVs over than 1 are crucial for the aroma of the sample. A larger OAV means a greater impact on the overall aroma (Steinhaus & Schieberle, 2007). Next, compounds with OAVs greater than 1 will be added to the non-flavored matrix according to the corresponding concentration to prepare the recombinant, and the difference between the aroma characteristics of the recombinant and the sample will be investigated. Thus, key odor active compounds will be first identified. At last, omission tests will be carried out by omitting the compounds (OAV ≥ 1) one by one to judge their contribution to certain aroma characteristics and verify the key odor active compounds. Recombination and omission test play an important role in the identification of key odor active compounds (Steinhaus & Schieberle, 2007). Molecular sensory sciences have been successfully applied in the identification of key odor active compounds of kinds of foods, like fruit, vegetable, etc (Steinhaus & Schieberle, 2007).

1.2.2. Electronic-nose (E-nose)

The term E-nose was firstly defined by Gardner and Bartlett in 1994 (Gardner & Bartlett, 1994). E-nose is an intelligent chemical array sensor system, comprised of an array of electronic chemical sensors with partial specificity and a pattern-recognition system, to mimic the process of the human nose when assessing the smell of the odorant molecules emitted from an object. The design of the working principles of the E-nose is to mimic human olfaction (Tan & Xu, 2020). As shown in the schematic diagram (Figure 2-4), the specific gas sensor arrays will first respond to the volatiles derived from the samples. According to the materials, there are several types of sensors including conducting polymers (CP), metal-oxide semiconductor (MOS), quartz crystal microbalance (QCM), and surface acoustic wave (SAW) sensors. Among these types of sensors, MOS sensors respond to a broad range of volatiles like aldehydes, alcohols, and ketones though they are less responsive to molecules like terpenes, aromatic compounds, or organic acids (Berna, 2010). Meanwhile, MOS E-noses have very high sensitivity, but limited sensing range, rapid response and recovery, but are susceptible to sulfur and acid, sensitive to high humidity (Wilson, 2012). MOS E -noses also have good reproducibility (precision) and limited manufacturing costs (Wilson, 2012). There is a wide range of application of MOS E-nose related to quality control, monitoring process, aging, geographical origin, adulteration, contamination, and spoilage (Berna, 2010). Thus, MOS E-nose was applied in this subject. The interpreted data will then be processed into pattern recognition algorithms, which could further play an important role to discriminate and classify the samples (Tan & Xu, 2020). Nowadays, E-nose has been applied successfully in many practices like the adulteration, classification, and authentication of food (olive oil, coffee, pepper, and tea) (Mildner-Szkudlarz & Jelen, 2010; Rodríguez et al., 2014; Kovacs et al., 2010). The disadvantage is that E-nose needs a long time of heating before the test procedure to reach the temperature of 150 to 400 °C.

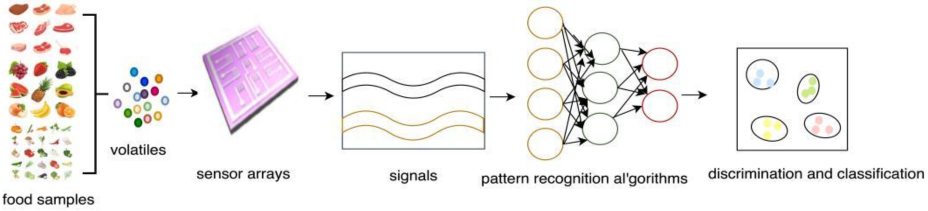


Figure 2-4 The working principle of E-nose

1.2.3. Analytical methods of aroma compounds

1.2.2.1 Sample preparation: solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) is based on the sorption of volatile and semi-volatile compounds released from the samples, on or into a polymeric material that coats a silica fiber (Figure 2-5) (Stashenko & Martínez, 2007). The equilibrium conditions can be described by the equation (2.1). The equilibrium is reached when a plateau on the extraction yield is reached, nevertheless, it is not necessary to work at equilibrium to have good and repeatability performance (Pawliszyn, 2012). At present, SPME has been applied in many fields like food, environment, and polymers, in view of its advantages of less sample volume, simple sample preparation, and solvent-free extraction (Stashenko & Martínez, 2007). HS-SPME was firstly introduced by Zhang and Pawliszyn in 1990s (Zhang & Pawliszyn, 1993). Since then it has become the most used mode of HS sampling technique. Being contacts less with the sample, it avoids the contamination or decomposition of the fiber coatings, and weakens matrix influences (Stashenko & Martínez, 2007).

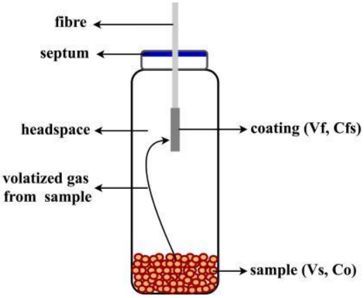


Figure 2-5 The working principle of HS-SPME

$$n = \frac{Kfs\,Vf\,Co\,Vs}{Kfs\,Vf + Khs\,Vh + Vs} \qquad (2.1)$$

n: amount of analyte extracted onto the coating
Vh: headspace and the matrix
Vf: volume of fibre coating
Vs: volume of sample
Kfs: fibre or sample distribution coefficient
Co: initial concentration of the analyte in the sample

Cf* , Ch* , Cs*: the equilibrium concentrations of the analyte in the coating, the headspace, and the matrix
Khs: Ch*/Cs*

1.2.2.2 Gas chromatographic separation

GC was invented by Martin and James in 1952 (Martin & James, 1952). After the injection of the sample in gaseous form, the separation occurs in the column, which represent the heart of the system. The column is a silica fused capillary covered by a stationary phases. The separation through the column occurs due to the partition of the analytes between the stationary phase and the carrier gas (Bartle & Myers, 2002).

1.2.2.3.Detection of compounds

1). MS

Through the measurement of mass-to-charge ratios of the ions from the gaseous sample, the mass spectrometer allows for qualitative and quantitative analysis. (Medhe, 2018). A mass spectrometer usually composed by five parts: I). a gas chromatograph or a direct insertion probe as an inlet; II). an ion source; III). one or several analysers; IV). a detector; V). a data processing system (Figure 2-6). During the mass spectrometric analysis, gas phase ions form firstly from the ionization of the compound. Secondly, the primary product ions would continue to fragment. Thirdly, the formed ions with different mass-to-charge ratios are separated in the mass analyser, and detected according to their abundance ratios. Finally, the spectrum is showed with a graph using mass-to-charge ratio as *x* axis and relative abundance as *y* axis (De Hoffmann & Stroobant, 2007).

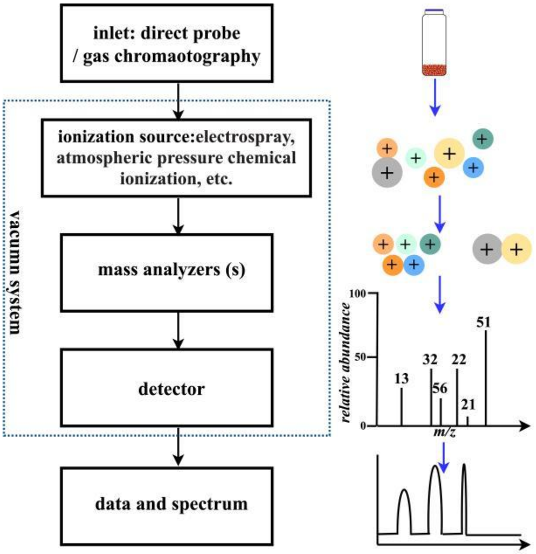


Figure 2-6 The working principle of Mass spectrometers

2). Tandem MS (MS/MS)

Tandem mass spectrometry was developed in the 1960s (McLafferty, 1981). There were two mass spectrometers in HS-SPME-GC-MS/MS, one (MS-I) responsible for the transmission of some ions, the other (MS-II) for the separation and detection of the ions that exit from MS-I (McLafferty, 1981). In this way, the information from the MS-II of a certain ion will assist in the identification of the corresponding compound by increasing the specificity. After years of development, the mass accuracy of MS/MS is improved to 1-5 ppm (Kind et al., 2018). And the reference database availability has been well organized for a broader identification (Kind et al., 2018). The sequential MS spectrometers make it possible to remove the matrix-arise interferences through the mass filters, thus reducing the detection limit and improving the sensitivity of detection. Nowadays, the application of MS/MS is more extensive, especially in targeted compounds analysis in food and environment areas. For example, the technique of HS-SPME-GC-MS/MS was used for the detection of 2-acetyl -1-pyrroline with a calibration range of 53 and 5380 pg/g, which was below its sensory threshold (Hopfer et al., 2016). The detection of chemical warfare agent and their degradation products in environmental samples using HS-SPME-GC-MS/MS could reach the limit of detection (LOD) to the range of 0.03 and 0.65 ppb (Nawała et al., 2016).

3). Ion mobility–mass spectrometry (IMS)

IMS was firstly developed by Barnes, Martin, and McDaniel in 1961 (Barnes et al., 1961). The working principle of IMS is shown in Figure 2-7. Firstly, the gaseous sample will be heated, vaporized, and transferred by the carrier gas into the ionization region. In the ionization area, the sample molecules and carrier gas molecules will be ionized to form various product ions. Secondly, these ions will travel through the periodically worked shutter grid, enter into the drift tube which is continuously supplied with drift gas. Thirdly, the mixed ions will arrive at the detector at different times, which are named drift times. The different drift times caused by the differential mobility of the ions will give information on the characteristics of the analyte. At last, the qualification and quantitation of the analytes will be achieved through the peak times and peak heights (Leonhardt, 2003). To avoid the problematic cross sensitivities caused by the humidity in ambient air, GC-columns are introduced with IMS in 1980s to pre-separate the complex compounds in the samples firstly (Baim, 1983). Preseparation by GC avoids clustering in the ionization region, improving selectivity, and the retention time obtained from this setup can be used as an additional parameter for the identification of the detected compounds.

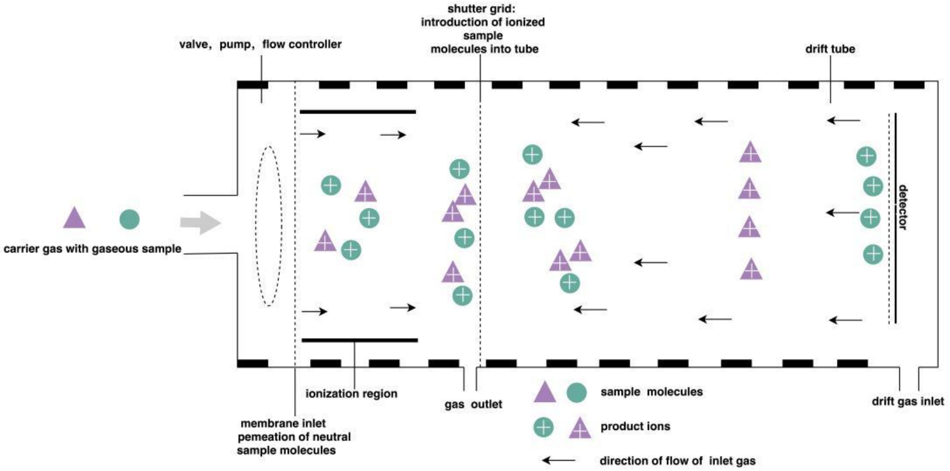


Figure 2-7 Schematic working principle of ion mobility spectrometry

HS-GC-IMS is manufactured by FlavourSpec®, Gesellschaft für Analytische Sensorsysteme mbH (G.A.S., Dortmund, Germany) and widely applied in the analysis of food aroma (Figure 2-8) (Gu et al., 2021). The technique of HS sampling has the advantages of avoiding direct sample probing and simplification of the complex sample matrix (Zhu & Chai, 2005). HS-GC-IMS shows advantages in aspects of fast detection speed, convenient operation, and portable equipment (Wang et al., 2020). Nowadays, HS-GC-IMS has been widely used in food aroma analysis including classification, freshness, and quality monitoring (Wang et al., 2020). For example, HS-GC-IMS was applied for the quality classification of virgin olive oils, discrimination of white and yellowed rice, fingerprinting of volatile compounds of Tricholoma matsutake Singer (Valli et al., 2020; Zhang et al., 2020; Li et al., 2019). Besides, a combination of HS-GC-IMS and HS-SPME-GC-MS was widely used to analyze the volatiles of food samples like *Tricholoma matsutake* Singer and Liuyang Douchi, in order to supplement the limitation of an incomplete database of GC-IMS (Zhang et al., 2020; Chen et al., 2021a).

Figure 2-8 The system of HS-GC-IMS

4). Olfactometry (O)

The analytical chemistry of aroma research is typically to extract, identify, and quantifier the various aroma compounds. However, the aroma perception is a result of aroma compounds and their interaction with our oral environment. The interaction is a dynamic process as the intensities and concentrations of the volatiles emanating from the samples may evolve over the time. Thus, the technique of olfactometry was created to capture the time – intensity patterns of the volatile compounds that was inhaled by the olfactory receptors (Berger, 2007). The review of the trials for olfactometers has been carried out in 1955 (Mateson, 1955). The principle of the apparatus is the observation of the odors in the odorivector that were extracted, confined, and diluted from the sample (Mateson, 1955). The hybrid GC(-MS) and O instrument combines the instrumental separation and human perception of the odors, building a relationship between the odors and the olfactory stimulus (Brattoli, 2011). Besides, GC(-MS)-O also can differentiate the volatiles into odor-active or non odor active, with the effect of their concentration and matrix (Zellner et al., 2008). The olfactometric ports, also called sniffing port, is usually made of glass or polytetrafluoroethylene cones. The outline of GC-O is dipicted in Figure 2-9, the sample gas is split at the end of the column and transported to the detector and the nose, separately. The transfer line is heated to protect semi-volatiles from condensation. The humid air is added to avoid nasal dryness of the panelists. The chromatogram (a), the olfactogram (b), and the overlay of the two traces (c) will be compared for the identification and odor description of the odorous compounds (Brattoli, 2011).

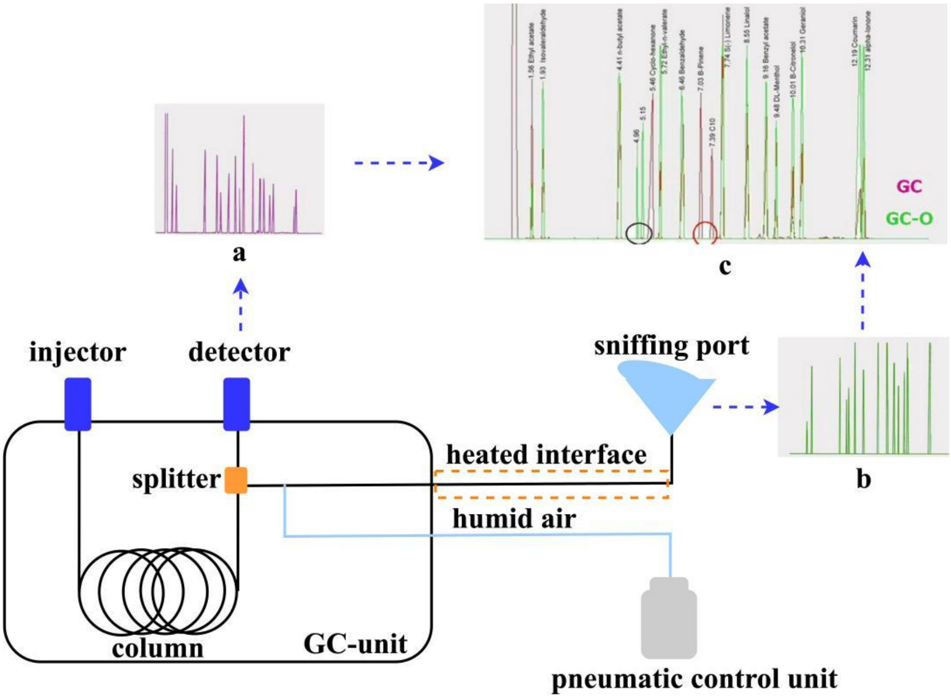


Figure 2-9 The working principle of GC-O

1.3. Research progress of jujube aroma

1.3.1. Fresh jujube aroma

The aroma profile of 22 kinds of fresh jujube that cultivated in different regions of China has been studied through the techniques of HS-SPME-GC-MS, E-nose, HS-GC-IMS, GC-O, and sensory analysis (Table 2-2). The volatiles of fresh jujube were identified as alcohols, acids, aldehydes, esters, ketones, and furans (Chen et al., 2018; Song et al., 2019). Aldehydes (54-78%) and acids (8-26%) were the main volatile components of fresh jujube (cv. Muzao, Yuanzao, Lizao, Xiangzao, Banzao, Jinsixiaozao, Pingdingdazao, Lingwuchangzao, and Junzao) (Chen et al., 2018; Song et al., 2019). The study on the aroma profile of winter jujube cultivated in Shandong Province, China, showed that aldehydes were the most dominant volatiles (57%), followed by acids (28%), and esters (12%) (Yang et al., 2019). Winter jujube, also known as "Dongzao", is the most prominent variety of table jujube, which is impressed by its fresh and green aroma characteristics. However, there was no systematic research carried out to clarify the volatile profiles of winter jujubes from different regions.

1.3.2. Red jujube aroma

The aroma characteristics of red jujube (cv. Tangzao; Muzao; Lizao; Qingrunhongzao; Jinsixiaozao; Youzao; and Yuzao from the production areas of Hebei, Shaanxi, and Xinjiang Province, China; cv. 'GAL', 'MSI', and 'PSI' from province of Alicante, Spain) have been studied through the techniques of HS-SPME-GC-MS, E-nose, GC-O, and molecular sensory sciences (Table 2-2) (Wang et al., 2018; Zhu & Xiao, 2018). The results showed the VOCs of (E)-2-hexenal, (E)-2-heptenal, (E)-2-octenal, hexanal, 2,3-butanedione, *β*-damascenone, ethyl hexanoate, 3-mercaptohexyl acetate, and 2,5-dimethylpyrazine were the key odor active compounds of the red jujubes (Wang et al., 2018; Zhu & Xiao, 2018). Besides, the sensory analysis highlighted the attributes of sweet and jujube ID were the strongest sensory characteristics (Zhu & Xiao, 2018). There are kinds of red jujubes cultivated in Xinjiang Province due to the region's climatic characteristics, but fewer of them have been studied in the aspect of cultivar-differed aroma characteristics.

1.3.3. Processed red jujube aroma

For the processing of jujube, there are kinds of red jujube-based products like liquor, bread, cakes, slices snack, tea, etc (Rashwan et al., 2020). In terms of slices snack and tea, red jujubes need to be dried and processed into jujube powder. At present, many studies have explored the influence of drying methods (50~95 °C) on the aroma of red jujube (Song et al., 2010; Wang et al., 2016). The aroma of jujube wine made from jujube fermentation has also been studied (Xia et al., 2020; Zhao et al., 2021). However, the aroma of thermal processing of red jujube associated with bread or cake has not been investigated yet.

Table 2-2 Research progress on jujube aroma

Usage	Cultivar	Region	Method	Identificaiton Results	Reference
table fruit	Muzao, Yuanzao, Lizao, Xiangzao, Banzao	Shanxi Province, China	HS-SPME/GC–MS; E-nose	sensory evaluation:sweet, floral, fruity, and green. VOCs: aldehydes(12), acids(10), esters(9), alcohols (5), ketones (4), furans (1), terpenoids (1), naphthalene (1), phenols (1), others (7).	Chen et al., 2015
	Dongzao	Shandong Province, China			
	Jinsixiaozao	Hebei Province, China			
	Pingdingdazao	Liaoning Province, China			
	Lingwuchangzao	Ningxia Province, China			

Junzao	Xinjiang Province, China			
Banzao, Dabailing, Jinsixiaozao, Huping, Lingbao	Shanxi Province, China	HS-SPME/GC-MS	VOCs: aldehydes (18), alcohols (2), ketones (3), acids (5), esters (5).	Wang et al., 2019
Yuanling, Jidan,Lizao, Baodeyouzao, Jinzao				
Junzao, Pingshunjunzao				
Xiangzao, Pozao, Neihuangbianhesuan				
Huizao, Junzao, Hamidazao, Dongzao	Xinjiang Province, China	HS-SPME/GC–MS; E-nose	VOCs: acids (14), aldehydes (14), esters (10), ketones (5), alcohols (2).	Liu et al., 2021
Junzao	Xinjiang Province, China	HS-SPME/GC–MS; E-nose	VOCs; alcohols (2), acids (9), aldehydes (5), esters (4), ketones (2), furan (1), phenol (1), others (3).	Song et al., 2019

dried fruit	Dongzao	Shandong Province, China	HS-GC-IMS	VOCs: alcohols(15), aldehydes (7), ketones (8), eaters (10), acids (2), pyrazines (3), furans(2), sulfides (4), others (2)	Yang et al., 2019
	Jinsixiaozao	Hunan Province, China			
	Tangzao; Muzao; Lizao; Qingrunhongzao	China	SDE-GC-MS; E-nose; GC-FID-O; Sensory analysis	VOCs: alcohols (6), aldehydes (7), ketones (8), esters (10),acids (2), pyrazines (3). Sensory evaluation: sweet, sour, green, fruity, roast, mellow. FD factors $\geqslant 4$: (E)-2-hexenal, (E)-2-heptenal, 2,3-butanedione, 2,5-dimethylpyrazine, β-damascenone	Wang et al., 2018
	Jinsixiaozao	Hebei Province, China	HS-SPME-GC-MS; GC-O; Sensory analysis	VOCs: alcohols (4), aldehydes (10), ketones (7), esters (4), acids(2), pyrazines (5), sulfides (2). KOAVs:hexanal, (E)-2-octenal, β-damascenone, ethyl hexanoate, 3-mercaptohexyl acetate, 2,5-dimethylpyrazine	Zhu&Xiao, 2018
	Youzao	Shaanxi Province, China			

		Yuzao	Xinjiang Province, China			
		‘Grande de;Albatera, ‘GAL’, ‘MSI’, and ‘PSI’’	province of Alicante, Spain	Sensory analysis;	Sensory evaluation: sourness, sweetness, bitterness, astringency, jujube-ID, vegetable, apple, fruity, hay-like, woody, burnt, and aftertaste	Wojdyło et al., 2016
	drying methods	Jinsixiaozao	Beijing, China	HS-SPME-GC-MS	VOCs: alcohols(7), aldehydes(12), ketones (5), esters (7), acids(9), alkanes (5), others (1)	Wang et al., 2016
processed fruit	drying methods	Junzao	Xinjiang Province, China	HS-SPME-GC/MS;MOS e-nose and flash GC e-nose	VOCs: alcohols (2), aldehydes (1), ketones (1), esters (1), acids(10), others (1)	Song et al., 2020
	drying methods	red jujube	Shandong Province, China	HS-SPME-GC-MS;	VOCS: acid (1), alkanes (18), ketones (4)	Bi et al., 2014

jujube brandy	red jujube	Hebei Province, China	HS-SPME-GC-O-MS	VOCs: alcohols (7), aldehydes (7), ketones(3), esters (23), acids (7), others(7)	Xia et al., 2020
jujube wine	red jujube	Xinjiang Province, China	GC-MS; Sensory analysis	VOCs: alcohols (8), aldehydes(1), ketones (1), esters (24), acids (6), others (2)	Zhao et al., 2021

2. Conclusion and future trends

Jujube has multiple bioactive compounds and a special aroma. There is a wide distribution of jujube and varieties of jujube cultivars for different usages in China. As a traditional homology resource of food and medicine, jujube is usually consumed directly or thermally processed. At present, no report focused on the research on the geographical distribution and varieties of the aroma of jujube and correlates their sensory attributes with chemical compositions. Moreover, the aroma of thermally processed products like jujube tea or jujube cake is pleasant and attractive. However, there is no relevant research to track and identify the key odor active compounds of thermally processed red jujube.

Therefore, to clarify and supplement the aroma profile of fresh jujube, red jujube, and thermally processed jujube, investigations of untargeted and targeted aroma profiles are necessary.

3

Chapter Ⅲ. Volatile profile characterization of winter jujube from different regions via HS-SPME-GC/MS and GC-IMS

The VOCs of fresh winter jujube was composed by alkanes, alcohols, esters, and amines. Geographic region and variety will cause the change of VOCs as reported for different varieties of red jujube. There were significant differences in contents of alcohols, acids and aromatic compounds among different red jujube varieties. However, there is no systematic study on the geographical differentiation of winter jujube, especially on the VOCs. The aim of this study was to investigate the aroma profile of winter jujubes from different regions.

Qiao, Y., Bi, J., Chen, Q., Wu, X., Gou, M., Hou, H., Jin, X., and Purcaro, G. (2021). Volatile Profile Characterization of Winter Jujube from Different Regions via HS-SPME-GC/MS and GC-IMS. *Journal of Food Quality*, 2021. https://doi.org/10.1155/2021/9958414.

Abstract

A combined untargeted and targeted approach was established for fingerprinting volatile organic compounds in winter jujubes from eight regions of China. Volatiles, including alcohols, aldehydes, acids, esters and alkenes were identified by gas chromatography-ion mobility spectrometry (GC-IMS). Benzyl alcohol, octanoic acid, 2-hexenal, linalool, 2-nonenal and ethyl decanoate were the most common compounds presented in all jujubes. Principal component analysis (PCA) from GC-IMS and untargeted E-nose showed that the main volatile organic compounds (VOCs) of most jujubes were similar. The volatile organic compounds of winter jujubes from Yuncheng city, Shanxi province and Akesu region, Xinjiang province were significantly different from those from other regions. 1-Penten-3-ol, ethyl hexanoate, methyl laurate, and 2-formyltoluene were the markers of XJAKS with green and fruity aroma, SXYC could be labeled by acetone and 2-methoxyphenol with woody and pungent aroma. GC-IMS was an effective method for the volatile fingerprinting of jujubes with high sensitivity and accuracy.

Keywords: Winter jujube, GC - IMS, Volatile markers, VIP scores

1. Introduction

Winter jujube (*Zizyphus jujuba Mill.* cv. Dongzao), is a unique late-ripening jujube cultivar that originated in China (Zhao et al., 2020). It is widely distributed in China, including Hebei, Shandong, Shannxi, Shanxi and Xinjiang province after years of domestication and cultivation (Zhou et al., 2019). As a representative variety of fresh jujube, it is recognized for its delicious taste and pleasant aroma (Zhao et al., 2020; Zhao et al., 2019). As a vital factor considered by consumers, sensory quality can be affected by aroma characteristics, which was correlated with VOCs. The VOCs of fresh winter jujube are composed of alkanes, alcohols, esters, and amines (Pu et al., 2011). Geographic region and variety will cause the change of VOCs as reported for different varieties of red jujube (Wojdyło et al., 2016; Chen et al., 2018). There are significant differences in the contents of alcohols, acids and aromatic compounds among different red jujube varieties (Chen et al., 2018). However, there is no systematic study on the geographical differentiation of winter jujube, especially on the VOCs.

At present, HS-SPME/GC-MS and E-nose are widely applied for the analysis of VOCs in jujube (Pu et al., 2011; Chen et al., 2018). HS-SPME/GC-MS combines the high separation ability of GC, the advantages of easy to use, automation, and solvent-free of SPME, and the superior identification ability of MS for VOCs (Luisa et al., 2019). E-nose distinguishes samples via the response to VOCs on different metal oxide sensors. However, it defects in the identification of differential components (Mohd et al., 2020). In contrast to these analytical methods, IMS is a complementary way to detect compounds with low concentration (ppbv levels) with the advantage of fast response (Karpas, 2014). In IMS, vapors from the sample are first transferred into the ionization region by the carrier gas. Secondly, the product ions formed from the interaction between neutral sample molecules and the buffer gas, or bath gas molecules in the drift tube will be transported by the electric field into the separation region. Thirdly, the product ion, characterized by different drift velocities will reach the detector at different times. Finally, the drift time is used for the qualitative analysis (Karpas, 2014). GC-IMS combines the separation characteristics of GC and the fast response of IMS (Wang et al., 2020). GC-IMS has been proved successful in regional identification and varieties classification based on the differentiation of VOCs (Wang et al., 2019; Pu et al., 2019; Li et al., 2019). GC-IMS was also used to monitor the changes of VOCs in winter jujube during cold storage (Yang et al., 2019). Besides, the combined analysis of GC-IMS and GC-MS has been successfully applied in the differentiation of VOCs from food matrices (Li et al., 2021a; Chen et al., 2021a; Chen et al. 2021b). However, few investigations on the fingerprinting of volatile profiles in winter jujubes from different regions have been done by GC-IMS.

Principal component analysis (PCA) effectively classifies samples using unsupervised statics and has been widely employed in chemometrics and bioinformatics (Chatterjee et al., 2018). Variable influence on projection (VIP) (greater than 1.0) has been applied to identify primary and specialized metabolites that are responsible for the discrimination of fruit and vegetables (Hoffmann

&Carvalho, 2017). PCA and VIP scores would effectively differentiate and select potential aroma maker of winter jujubes.

In this study, the geographic differentiation of winter jujubes was investigated by GC-IMS and E-nose based on VOCs. Furthermore, the potential markers of winter jujube from each specific region were identified and quantified by GC-IMS and GC-MS.

2. Materials and methods

2.1. Winter jujube preparation and chemicals

Winter jujubes from eight regions were purchased from China's famous jujube trading market (Beiyuanchun Jujube Market in Xinjiang Province, Xinzheng Jujube Market in Henan Province, and Cuierzhuang Jujube Market in Hebei Province). The information on jujube samples is shown in Table 3-1. Winter jujube samples were collected 50 kg from each region. Fresh fruits free from pests and diseases were washed, pits removed, sliced, and frozen by liquid nitrogen, then stored at -40 °C for testing as soon as possible.

Table 3-1 Geographical distribution information of winter jujubes from eight regions

Abbreviation	Geographical origin	Longitude and latitude
HBHH	Hebei Province, Huanghua City	E117.30, N 41.03
SDZH	Shandong Province, Zhanhua City	E 118.14, N 37.7
SDYT	Shandong Province, Yantai City	E 121.17, N 36.76
SXYC	Shanxi Province, Yuncheng City	E 110.15, N34. 35
SXLY	Shanxi Province, Linyi City	E110.77, N35.15
SNXDL	Shannxi Province, Dali City	E 109.93, N 34.80
XJKEL	Xinjiang Uygur Autonomous Region, Kuerle City	E 86.06, N 41.68
XJAKS	Xinjiang Uygur Autonomous Region, Akesu	E 80.29, N 41.15

The mixture of C4 - C20 alkanes was purchased from O2si Smart Solution (Charleston, West Virginia, USA), cyclohexanone was purchased from Genenode Trading Co. Ltd (Beijing, China), n-hexane was purchased from Hushi Co. Ltd (Shanghai, China). Standards used in this project were as follows: benzyl alcohol, octanoic acid, 2-hexenal, 2-heptenal, linalool, methyl myristoleate, 2-nonenal, styrene, hexanoic acid, hexanal, ethyl decanoate, pentanoic acid, 1-penten-3-ol, 1-octen-3-ol, E-2-hexen-1-ol, linalool, butanoic acid, pentanoic acid, heptanoic acid, (E)-3-hexanoic acid, octanoic acid, nonanoic acid, acetone, 3-pentanone, 2-hexanone, acetoin, 6-methyl-5-hepten-2-one, (E)-4-undecenal, butanal, 2-hexenal, E-2-heptanal, heptanal, 2-heptenal, 2,4-heptadienal, 2-nonenal, 2-decenal, 3-buten-2-one, ethyl hexanoate, methyl decanoate, methyl laurate, methyl myristoleate, limonene, 2-formyltoluene, 2-formylphenol, 2-nitrophenetole, 2-nitro-phenol, 2-methoxyphenol, and 2-pentyl furan were all purchased from MilliporeSigma (St. Louis, MO, USA).

2.2. Sample preparation

About 100 g of frozen winter jujube slices were ground for 60 s with Joyoung juicer (JYL-CO20, Joyoung Co., Ltd., Shandong, China). Accurate 2.0 g of winter jujube pulp was put into a 20 mL vial sealed with a magnetic screw cap and septum before testing.

2.3. GC-IMS analysis

A FlavourSpec instrument (G.A.S. Gesellschaft für analytische Sensorsysteme mbH Dortmund, Germany) was used for GC-IMS analysis. Procedures were referred to in previous studies with minor modifications (Gerhardt et al., 2019). Briefly, the sample vial was incubated at 50 ° C for 20 min, then 500 μL of headspace was injected at 85°C in splitless mode. Tritium (6.5 KeV) was used as the ionization source in this project. A FS-SE-54-CB-1 (15 m × 0.53 mm ID) column was used for separation at 60 °C. Linear pressure program of the column was as follows: 2 mL/min for 2 min, ramped up to 10 mL/min over 8 min, then reached to 100 mL/min over 10 min, at last, got to150 mL/min over 5 min. Nitrogen of 99.99% purity was the drift gas at a flow rate of 150 mL/min and the drift tube was operated at 45 ° C. All standards were run under the same test procedure to supplement GC×IMS Library Search for qualitative analysis.

The spectrogram was analyzed by Laboratory Analytical Viewer (LAV) where retention time and drift time were analyzed for the qualification of VOCs. The reporter plug-in was used to compare spectrogram differences among samples from the two-dimensional and three-dimensional view. Gallery Plot plug-in was used to compare the differences between volatile fingerprints visually. PCA plug -in was used for classification analysis.

2.4. E-Nose analysis

A commercial PEN 3.5 E-Nose (Airsense Analytics, GmBH, Schwerin, Germany) containing ten metal-oxide semiconductors was used to distinguish the overall flavor perception of winter jujubes from eight regions. The detailed procedure of sample preparation was referred to Chen et al. (Chen et al., 2018). Sample preparation was the same as that in GC-IMS analysis.

2.5. HS-SPME GC-MS analysis

HS-SPME conditions and GC-MS analysis were referred to Chen et al. with minor modifications (Chen et al., 2018). The vials (the same as those in GC-IMS analysis) were equilibrated at 50 °C for 40 min.

Polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber was used for extraction and desorbed at 250 °C for 3 min in splitless mode. DB-WAX capillary column (30 m × 0.25mm × 0.25 μm) was used for separation. The oven temperature program was as follows: 40 °C for 3 min, heated to 120 °C (5 °C/min), then rose to 200 °C (10 °C/min) and held at 200 °C for 5 min. Helium was the carrier gas at a flow rate of 1.0 mL/min. Electron Impact mode with the ion source temperature set at 200 °C and the ionization energy of 70 eV was performed in the MS detector. The acquisition was in full-scan mode and mass acquisition range was 35-550 *m/z*.

The standards were qualitatively analyzed under HS/SPME GC-MS analysis. The retention indeces (RI) of the compounds were calculated based on the retention time of C4 - C20 alkanes mixture and used as additional support for the identification of compounds. A 2 mg/L solution of cyclohexanone as the optimized internal standard was used for the relative quantitative analysis of VOCs. NIST 17 database was used for the identification of the compounds.

2.6. Statistical procedures

Data standardization was performed using SPSS 13.0 software (SPSS Inc, Chicago, IL). MetaboAnalyst was used for bi-plot and calculation of LDA analysis and VIP scores. (https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml). Morpheus was applied for the correlation analysis by heatmap. (https://software.broadinstitute.org/ morpheus/). Each sample was repeated in triplicate.

3. Results and discussion

3.1. Determination of VOCs from eight regions of winter jujube by GC-IMS

VOCs of winter jujube samples from eight regions of China were analyzed by GC -IMS, the result was shown in Figure 3-1. The red baseline on the left is the reaction ion peak (RIP). RIP represents the total number of ions in the ionization chamber, described as $H+(H_2O)n$ (n represents the number of water molecules) (Gerhardt et al., 2019). The points on the right of RIP represent the signal (monomer, dimer and even trimer) of volatiles extracted from the samples. Most of the signals appeared in the retention time of 100-600 s and the drift time of 6.0-14.0 ms. The red color represents the higher signal intensity (higher concentration) of the substance, while the white color indicated weaker intensity (lower concentration) (Arroyo-Manzanares et al., 2018). In IMS, the formation of product ions mainly depends on the affinity of analytes to protons. Product ions could also be affected by the concentration of the analyte, the chemical properties, and the temperature of the drift tube (Gerhardt et al., 2019).

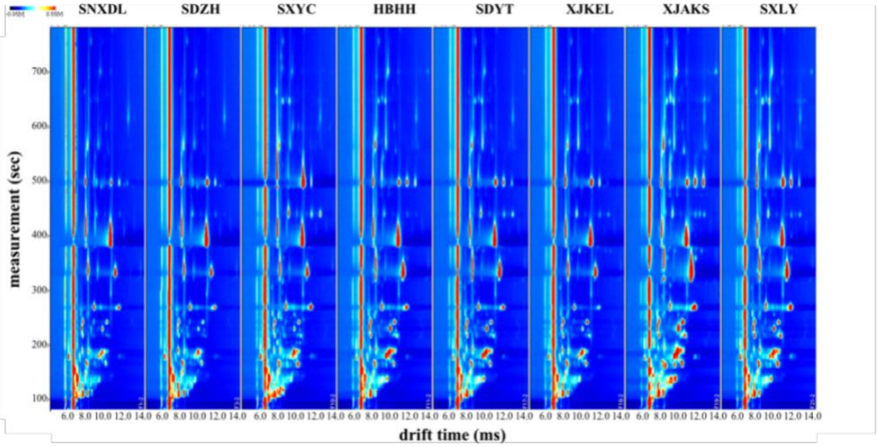


Figure 3-1 2D topographic plots of VOCs from eight regions of winter jujube

Varieties of fruits have distinct aroma depending on the composition, concentration, aroma description, and odor thresholds of VOCs (El Hadi et al., 2013). The composition of VOCs varied in winter jujubes from different regions. In order to identify the specific differences and make a quantitative comparison, all peaks were identified and numbered for fingerprint comparison. The direct comparison of the components in each sample is shown in Figure 3-2. VOCs in the black frame were the common components among eight regions of winter jujubes. VOCs in the yellow and red frames were the ones present only in SXYC and XJAKS. VOCs in the green frame were the different ones in eight regions of winter jujube. The corresponding data of retention time (RT) and drift time (DT) are presented in Table 3-2.

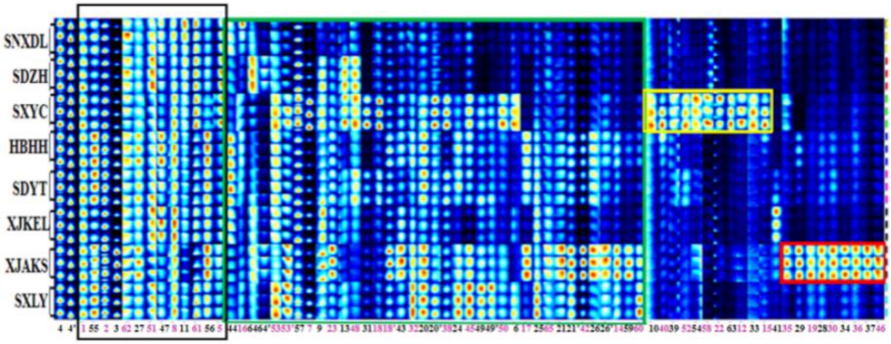


Figure 3-2 VOCs qualitative comparisons of eight regions of winter jujube by GC-IMS (The X-axis represented the code of the compound, corresponding to the information in Table 3-2)

Table 3-2 Volatile organic compounds identified by GC-IMS

No.	Compound	CAS	Fomula	Molecular weight	RI	Rt [sec]	Dt [RIPrel]	Identificaiton Approach
1	benzyl alcohol (monomer)	100-51-6	C_7H_8O	108	1090	758.55	1.21	RI, DT,Std
2	Unknown (monomer)	/	/	/	987	568.62	1.17	RI, DT
3	hexanoic acid (monomer)	142-62-1	$C_6H_{12}O_2$	116	989	573.49	1.30	RI, DT,Std
4	cyclohexanone (monomer)	108-94-1	$C_6H_{10}O$	98	904	410.86	1.15	RI, DT,Std
4'	cyclohexanone (dimer)	108-94-1	$C_6H_{10}O$	98	904	402.28	1.45	RI, DT,Std
5	hexanal (monomer)	66-25-1	$C_6H_{12}O$	100	796	269.29	1.56	RI, DT,Std
6	Unknown (monomer)	/	/	/	768	242.96	1.11	RI, DT
7	2,4-heptadienal (monomer)	5910-85-0	$C_7H_{10}O$	110	1427	242.38	1.41	RI, DT,Std

8	2-nonenal (monomer)	2463-53-8	C9H16O	140	687	181.34	1.07	RI, DT,,Std
9	3-buten-2-one (monomer)	79-77-6	C13H20O	192	1914	179.98	1.30	RI, DT,Std
10	Acetone (monomer)	67-64-1	C3H6O	58	814	110.75	1.11	RI, DT,Std
11	Styrene (monomer)	100-42-5	C8H8	104	602	140.40	1.05	RI, DT,Std
12	Unknown (monomer)	/	/	/	596	137.86	1.32	RI, DT
13	E-2-heptenal (monomer)	18829-55-5	C7H12O	112	570	127.33	1.04	RI, DT,Std
14	Unknown (monomer)	/	/	/	629	152.29	1.16	RI, DT
15	Unknown (monomer)	/	/	/	739	218.98	1.22	RI, DT
16	1-octen-3-ol (monomer)	3391-86-4	C8H16O	128	1349	226.98	1.15	RI, DT,Std
17	Unknown (monomer)	/	/	/	1042	664.75	1.14	RI, DT

18	limonene (monomer)	138-86-3	$C_{10}H_{16}$	136	1032	647.98	1.21	RI, DT,Std
18'	limonene (dimer)	138-86-3	$C_{10}H_{17}$	136	1032	647.00	1.30	RI, DT,Std
19	ethyl hexanoate (monomer)	123-66-0	$C_8H_{16}O_2$	144	1405	600.99	1.39	RI, DT,Std
20	Unknown (monomer)	/	/	/	961	513.04	1.15	RI, DT
20'	Unknown (dimer)	/	/	/	958	507.19	1.47	RI, DT
21	Unknown (monomer)	/	/	/	924	443.23	1.28	RI, DT
21'	Unknown (dimer)	/	/	/	922	440.50	1.67	RI, DT
22	Unknown (monomer)	/	/	/	786	259.34	1.09	RI, DT
23	nonanoic acid (monomer)	112-05-0	$C_9H_{18}O_2$	158	2083	256.62	1.36	RI, DT,Std
24	butanoic acid (monomer)	107-92-6	$C_4H_8O_2$	88	1538	218.01	1.12	RI, DT,Std
25	Unknown (monomer)	/	/	/	737	217.42	1.43	RI, DT

26	Unknown (monomer)	/	/	/	654	164.18	1.17	RI, DT
26'	Unknown (dimer)	/	/	/	655	164.97	1.40	RI, DT
27	2-heptenal (monomer)	2463-63-0	C7H12O	112	663	169.06	1.15	RI, DT,Std
28	2-formyltoluene (monomer)	529-20-4	C8H8O	120	1555	121.67	1.19	RI, DT,Std
29	1-penten-3-ol (monomer)	616-25-1	C5H10O	86	1134	122.26	1.03	RI, DT,Std
30	2-formylphenol (monomer)	90-02-8	C7H6O2	122	1542	116.99	1.22	RI, DT,Std
31	Unknown (monomer)	/	/	/	740	219.37	1.40	RI, DT
32	Unknown (monomer)	/	/	/	780	254.27	1.16	RI, DT
33	Unknown (monomer)	/	/	/	721	205.33	1.14	RI, DT
34	(E)-4-Undecenal (monomer)	68820-35-9	C11H20O	168	2719	203.77	1.43	RI, DT,Std

35	3-hexenoic acid, (E)- (monomer)	1577-18-0	C6H10O2	114	1876	730.66	1.19	RI, DT,Std
36	methyl laurate (monomer)	111-82-0	C13H26O2	214	1765	709.01	1.21	RI, DT,Std
37	2-pentylfuran (monomer)	3777-69-3	C9H14O	138	1218	317.26	1.21	RI, DT,Std
38	Unknown (monomer)	/	/	/	503	104.13	1.04	RI, DT
39	6-methyl-5-hepten-2-one	110-93-0	C8H14O	126	1335	652.47	1.26	RI, DT,Std
40	Unknown (monomer)	/	/	/	953	498.41	1.66	RI, DT
41	Unknown (monomer)	/	/	/	699	189.54	1.39	RI, DT
42'	Unknown (dimer)	/	/	/	699	189.54	1.42	RI, DT
43	methyl decanoate (monomer)	110-42-9	C11H22O2	186	1613	185.05	1.33	RI, DT,Std
44	2-hexanone (monomer)	591-78-6	C6H12O	100	1087	260.91	1.18	RI, DT,Std
45	Unknown (monomer)	/	/	/	954	499.97	1.56	RI, DT

46	Heptanal (monomer)	111-71-7	C7H14O	114	1280	373.42	1.32	RI, DT,Std
47	methyl myristoleate (monomer)	56219-06-8	C15H28O2	240	670	172.37	1.08	RI, DT,Std
48	2-decenal (monomer)	3913-71-1	C10H18O	154	1638	171.40	1.36	RI, DT,Std
49	Unknown (monomer)	/	/	/	1062	702.38	1.33	RI, DT
49'	Unknown (dimer)	/	/	/	1061	701.22	1.81	RI, DT
50	Unknown (monomer)	/	/	/	988	571.74	1.25	RI, DT
51	Linalool (monomer)	78-70-6	C10H18O	154	981	556.52	1.42	RI, DT,Std
52	phenol, 2-nitro- (monomer)	88-75-5	C6H5NO3	139	1810	359.18	1.57	RI, DT,Std
53	E-2-hexen-1-ol (monomer)	928-95-0	C6H12O	100	1361	346.71	1.18	RI, DT,Std
53'	E-2-hexen-1-ol (dimer)	928-95-0	C6H12O	100	1361	344.37	1.51	RI, DT,Std

54	2-methoxyphenol (monomer)	90-05-1	C7H8O2	124	1831	309.46	1.25	RI, DT,Std
55	octanoic acid (monomer)	124-07-2	C8H16O2	144	756	232.43	1.10	RI, DT,Std
56	pentanoic acid (monomer)	109-52-4	C5H10O2	102	754	231.26	1.35	RI, DT,Std
57	3-pentanone (monomer)	96-22-0	C5H10O	86	898	188.76	1.35	RI, DT,Std
58	2-nitrophenetole (monomer)	610-67-3	C8H9NO3	167	1651	162.82	1.19	RI, DT,Std
59	Unknown (monomer)	/	/	164	652	163.40	1.31	RI, DT
60	Acetoin (monomer)	513-86-0	C4H8O2	88	1120	204.55	1.05	RI, DT,Std
61	ethyl decanoate (monomer)	110-38-3	C12H24O2	200	682	179.01	0.94	RI, DT,Std
62	2-hexenal (monomer)	505-57-7	C6H10O	98	633	154.04	1.02	RI, DT,Std
63	Unknown (monomer)	/	/	/	525	111.14	1.17	RI, DT
64	butanal (monomer)	123-72-8	C4H8O	72	568	126.55	1.10	RI, DT,Std

64'	butanal (dimer)	123-72-9	C4H9O	72	864	124.99	1.28	RI, DT,Std
65	Unknown (monomer)	/	/	/	537	115.24	1.15	RI, DT

Common compounds included alcohols, aldehydes, acids, esters and alkenes (Figure 3-2). In particular, benzyl alcohol, octanoic acid, 2-hexenal, 2-heptenal, linalool, methyl myristoleate, 2-nonenal, styrene, hexanoic acid, hexanal, ethyl decanoate, and pentanoic acid presented small concentration difference (Figure 3-1). These identified volatiles and their aroma characteristics are important for the whole aroma of winter jujube.

Alcohols were formed by sugar catabolism and/or acid degradation in the food matrix (Issa-Issa et al., 2020). Benzyl alcohol contributes to a slightly fruity aroma, while linalool, a monoterpenoid, contributes to floral, lavender, lemon and rose flavor (Issa-Issa et al., 2020; Yuan et al., 2016). Aldehydes were thought to be mainly produced via lipid oxidation and decomposition, contributing most to the overall aroma among all categories because of relatively low odor thresholds. Hexanal derived from linoleic acid contributes to the flavor of fruity, grass, and green, with a low odor threshold of 1.1 ng/L (Hu et al., 2020). 2-Nonenal is a plant metabolite and derives from linoleate decomposition, with a flavor of paper (Hu et al., 2020). 2-Heptenal is a plant metabolite with a soapy and fatty aroma and also exists in white pomelo peel (Mayr, 2015). Acids like octanoic acid, hexanoic acid, and pentanoic acid might be related to cheese aroma (Tian et al., 2019). Ethyl decanoate is present in fruit like cherry and pineapple with fruity aroma (Arancha & María, 2020). Styrene was reported to have a sweet, balsamic, almost floral odor that was extremely penetrating (Burdock, 2010). Esters endow fruity aroma to fruits and methyl myristoleate was found to be part of the aroma of honey and iris (Qin, 2017). Mutual comprehensive effect of VOCs is the basis of the formation of the overall aroma of winter jujubes. Most of these common volatiles were found to be aroma attributes of fruity, grass or green, which was in agreement with Pu et al. (Pu et al., 2011).

As is shown in Figure 3-3, PCA of GC-IMS data showed SXYC and XJAKS were individually apart from the other winter jujubes. All the jujube studied in this study were distributed in the middle latitude of the northern hemisphere, among which SXYC has the smallest latitude and XJAKS has the smallest longitude (Table 3-1). The special geographical location and associated climatic factors of SXYC and XJAKS might be the reason why the jujube aroma of these two regions was different from others. SXYC and XJAKS had their own specific VOCs that are framed with yellow and red color separately in Figure 3-2. The substances identified in the yellow frame, including acetone, 6-methyl-5-hepten-2-one, 2-nitrophenol, 2-methoxyphenol, 2-nitrophenetole, were the specific chemicals in SXYC. XJAKS was characterized by compounds in the red frame, including (E)-3-hexenoic acid, 1-penten-3-ol, ethyl hexanoate, 2-methyl-benzaldehyde, 2-hydroxy-benzaldehyde, trans-4-undecenal, methyl laurate, 2-pentyl-furan and heptanal. The volatile markers of SXYC and XJAKS would be confirmed by the correlation analysis between E-nose and the relative quantitative results of GC-MS for the discriminating components in the following parts.

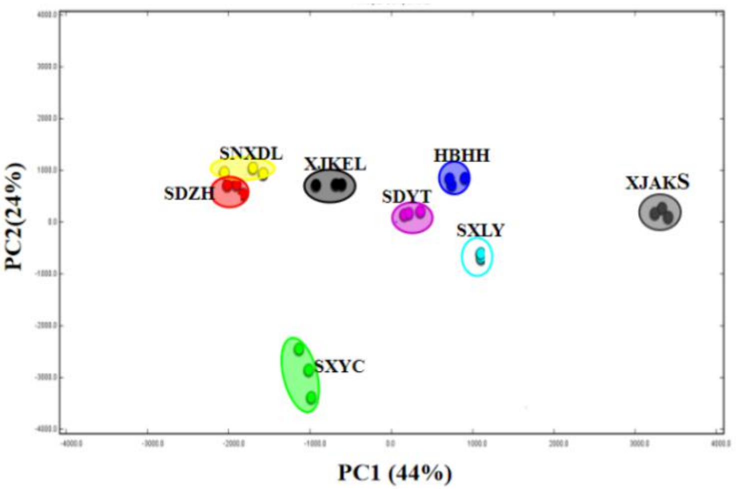
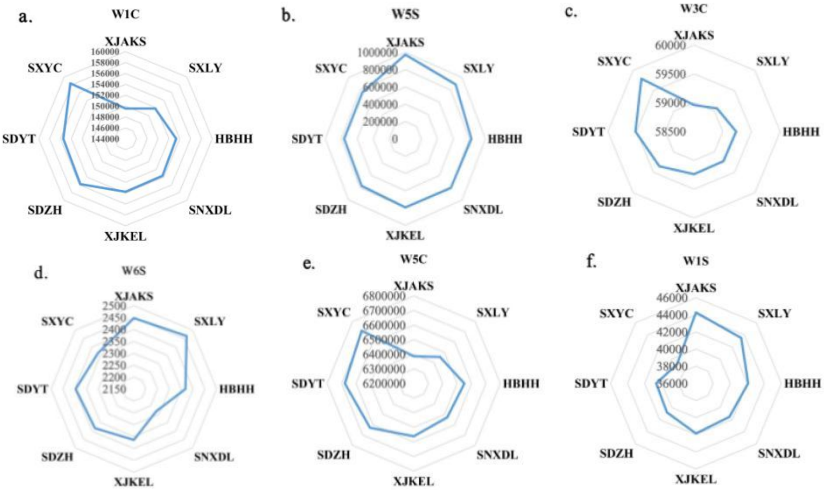


Figure 3-3 Principle component analysis (PCA) plot of eight regions of winter jujube

3.2. E-nose analysis

Aroma characteristics and PCA results based on E-nose of winter jujubes are shown in Figure 3-4 and Figure 3-5. Sensors and response characteristics of E-nose are shown in Table 3-3. E-nose results were not specific information on sample composition but rather a simple fingerprint through pattern recognition.



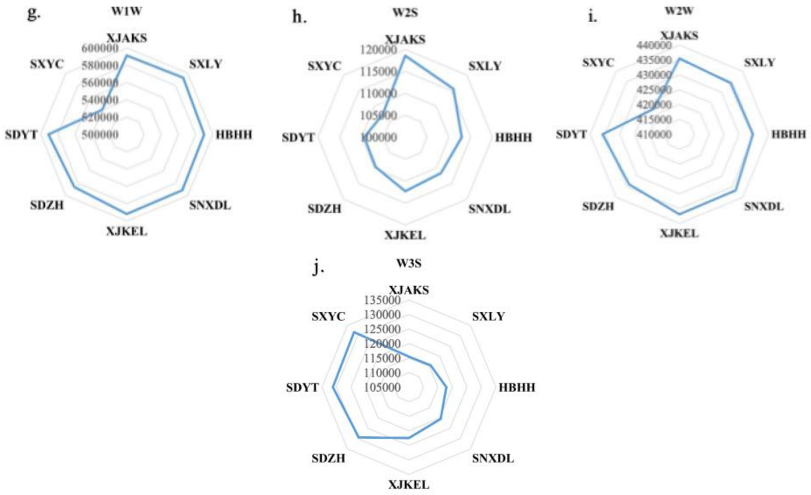


Figure 3-4 Response intensities of different sensors to aroma characteristics of different regions of winter jujube. (a-j represent the response on different sensors)

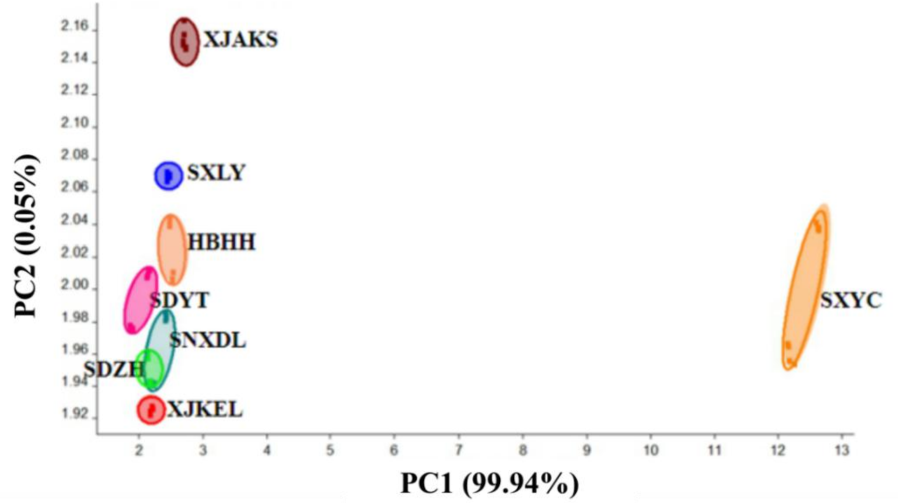


Figure 3-5 Principle component analysis (PCA) results of eight regions of winter jujubes differentiated by E-nose

Table 3-3 Sensors and response characteristics of E-nose

Number	Sensors	Response characteristics
1	W1C	aromatic compounds
2	W5S	nitroxide
3	W3C	ammonia and aromatic components
4	W6S	hydrogen selective
5	W5C	alkanes and aromatic components
6	W1S	methane
7	W1W	sulfides
8	W2S	ethyl alcohol
9	W2W	aromatic components and organic sulfide
10	W3S	alkanes

Figure 3-4(b), (g), (i) shows that VOCs of SNXDL, SDZH, HBHH, SDYT, XJKEL and SXLY generated almost the same tendency on sensors W5S, W1W and W2W. Aroma of SXYC had the highest response value on sensors of W1C, W3C and W5C (Figure 3-4 (a), (c), (e)). XJAKS had the highest response value on sensors of W5S, W1S, and W2S inferred from Figure 3-4 (b), (f), (h). PCA results (Figure 3-5) based on Figure 3-4 showed SNXDL, SDZH, HBHH, SDYT, XJKEL, and SXLY relatively clustered. SXYC and XJAKS were far away from others. The classification results of aroma characteristics obtained by E-nose (Figure 3-5) showed the same trend as those obtained by GC-IMS (Figure 3-3). The classification results of the two technologies strongly indicated that GC-IMS and E-nose had advantages in rapid classification and accuracy. Although targeted and qualitative analysis of winter jujubes was performed well by GC-IMS, the identification and quantitation of potential contributed markers in different winter jujubes were further combined with GC-MS analysis.

3.3. Potential markers analysis

HS-SPME/GC-MS was performed targeting the different VOCs (volatiles in the green, yellow and red frame in Figure 3-2 of winter jujubes. The results are shown in Table 3-4. Partial least squares discriminant analysis (PLS-DA) assesses the relationship between a descriptor matrix X and a response matrix Y in a supervised way (Hoffmann & Carvalho, 2017). The differential variations were amplified to illustrate the relationships between the groups in the bi-plot. Identification of the most important variables for the prediction ability of the PLS-DA model is generally performed by analyzing the regression coefficients. VIP scores provide information about the importance of each variable used in the grouping model of PLS-DA (Wold et al., 2001). VIP scores greater than 1.0 are always treated as the discriminating factor. PLS-DA has been applied to identify primary and specialized metabolites that are responsible for the discrimination of fruit and vegetables (Villa et al., 2018).

Table 3-4 Volatile organic compounds of winter jujubes quantified by HS/SPME GC-MS

					Concentration (mg/kg)								
	Compound	CAS	RI cal	RI lit	SNXDL	SDZH	XJAKS	HBHH	SDYT	XJKEL	SXYC	SXLY	Aroma description
Alcohols (3)	1-penten-3-ol*	616-25-1	1162	1148	6.11±0.33c	2.28±0.23c	52.08±5.74a	3.45±0.65c	1.75±0.20c	2.03±0.50c	30.7±3.12b	5.21±0.14c	grassy,green
	1-octen-3-ol	3391-86-4	1455	1449	8.06±0.13c	10.85±1.13b	0.12±0.02e	4.34±0.14d	0.44±0.01e	18.63±2.01a	nd.	0.45±0.02e	cucumber, earth, fat, floral, mushroom
	E-2-hexen-1-ol	928-95-0	1420	1409	8.98±0.45a	7.46±0.45ab	nd.	nd.	5.64±0.22b	8.76±3.26a	8.70±1.34c	nd.	blue cheese, vegetable

	Total				23.15±0.91	20.59±1.81	52.20±5.76	7.79±0.79	7.83±0.43	29.42±5.77	39.40±4.46	5.66±0.16	
Acids (3)	butanoic acid	107-92-6	1634	1623	nd.	3.98±0.22c	16.56±2.23a	5.65±1.21b	1.03±0.02d	0.45±0.02d	nd.	nd.	butter, cheese, sour
	3-hexenoic acid, (E)-	1577-18-0	1929	1948	0.70±0.11d	0.78±0.11d	5.19±0.11bc	nd.	3.00±0.23bc	36.11±7.89a	8.28±2.33b	nd.	fruit
	nonanoic acid	112-05-0	2174	2171	3.45±0.06c	0.22±0.01d	9.43±1.01b	0.43±0.01d	4.88±0.12c	7.70±2.12b	9.34±1.30b	20.43±3.23a	fat, green, sour
	Total				4.15±0.17	4.98±0.34	31.18±3.35	6.08±1.22	8.91±0.37	44.26±10.03	17.62±1.02	20.43±3.23	
Ketones	acetone*	67-	825	816	1.39±0.32	nd.	nd.	5.45±0.12	7.99±0.43	2.02±0.11	30.78±4.7	nd.	Pungent

(6)		64-1			c			b	b	c	8a		
	3-pentanone*	96-22-0	975	956	25.43±2.45b	38.80±3.15a	0.34±0.02d	nd.	1.60±0.05d	13.95±3.14c	10.84±1.23d	nd.	acetone-like
	2-hexanone	591-78-6	1124	1100	4.56±0.82b	4.34±0.82b	nd.	nd.	nd.	nd.	4.45±0.21b	19.16±3.02a	--
	acetoin	513-86-0	1272	1291	5.45±0.90b	2.22±0.20c	9.43±0.20a	nd.	4.88±0.32	nd.	nd.	nd.	butter, creamy, green pepper
	6-methyl-5-hepten-2-one	110-93-0	1548	1341	nd.	nd.	nd.	nd.	1.60±0.12b	nd.	3.84±0.22	nd.	citrus, mushroom, pepper, rubber, strawberry
	3-buten-2-one	79-77-6	1947	1958	1.39±0.12cd	6.10±0.12b	nd.	5.45±0.12b	8.99±1.11a	2.02±0.34c	0.88±0.09d	nd.	pungent odor

	Total				38.22±4.61	51.46±4.29	9.77±0.22	10.9±0.24	25.06±2.03	17.99±3.59	50.79±6.53	19.16±3.02	
Aldehydes (5)	butanal	123-72-8	898	867	1.39±0.22 bc	1.11±0.22 bc	1.37±0.22 bc	nd.	8.99±1.52 ab	2.02±0.52 c	0.88±0.09 d	nd.	pungent odor
	heptanal	111-71-7	1194	1327	0.97±0.11 d	nd.	3.43±0.21 c	nd.	nd.	9.43±0.21 b	16.43±2.11a	nd.	citrus, fat, green, nut
	2,4-heptadienal	5910-85-0	1490	1468	2.45±0.04 d	nd.	2.17±0.04 d	9.36±2.04 bc	9.87±1.23 ab	7.70±1.23 c	11.39±1.12a	nd.	--
	2-decenal	3913-71-1	1645	1659	0.23±0.01 d	4.34±0.11 c	6.70±0.31 b	0.54±0.11 d	nd.	nd.	4.45±0.32 c	19.16±1.52a	fat, fish, orange
	(E)-4-undecenal	68820-35-	2992	/	0.87±0.02 d	1.70±0.02 c	4.34±0.02 b	nd.	5.65±0.34 a	1.03±0.31 d	0.45±0.02 e	nd.	--

		9											
	Total				5.91±0.4	7.15±0.35	18.01±0.8	9.9±2.15	24.51±3.09	20.18±2.27	33.6±3.66	19.16±1.52	
Esters (3)	ethyl hexanoate	123-66-0	1435	1240	7.03±1.01c	1.84±0.41f	33.22±0.41a	4.56±0.52d	nd.	3.58±0.37e	8.67±0.69b	4.49±0.21de	apple peel, overripe fruit, pineapple
	methyl decanoate	110-42-9	1640	1633	16.34±1.23c	0.11±0.01e	0.09±0.01e	7.98±2.01d	30.47±3.23a	23.52±2.89b	5.51±2.11d	0.34±0.04e	a constituent of many plants
	methyl laurate*	111-82-0	1805	1805	1.03±0.01c	0.45±0.01c	34.43±3.01b	9.43±2.32a	0.43±0.01c	nd.	7.70±0.21a	9.34±2.33b	exist in melon, pineapple and other fruits
	Total				24.4±2.4	2.4±0.43	67.74±3.43	21.97±4.85	30.9±3.24	27.1±3.26	21.88±3.01	14.17±2.58	

Alkenes (1)	limonene	138-86-3	1192	1189	0.98±0.05cd	18.80±2.05a	0.34±0.05d	4.34±0.35c	1.60±0.32cd	13.95±4.34b	10.84±1.87b	1.45±0.05cd	lemon like aroma
	2-formyltoluene*	529-20-4	1646	1621	nd.	nd.	19.02±2.02a	0.12±0.02c	nd.	0.44±0.09c	8.63±1.98b	nd.	cherries and bitter almond
Benzenes (5)	2-formylphenol	90-02-8	1679	1636	0.44±0.02d	nd.	7.70±1.01b	2.17±0.03c	9.36±1.23a	nd.	2.43±0.10c	nd.	almond, pungent, spice
	2-nitrophenetole	610-67-3	1816	/	4.56±0.21b	4.34±0.21b	nd.	nd.	nd.	nd.	17.70±0.23a	nd.	--
	2-nitro-phenol	88-75-5	1819	1812	0.34±0.01e	nd.	nd.	7.98±0.10c	10.47±0.02a	3.52±0.04d	8.67±0.21b	nd.	peculiar sweet smell

	2-methoxyphenol*	90-05-1	1866	1862	5.45±0.32 b	2.22±0.30 cd	1.43±0.21 d	nd.	4.88±1.31 b	nd.	28.63±1.31a	2.89±0.14 c	burnt, phenol, wood
	Total				10.79±0.56	6.56±0.51	4.88±2.27	10.27±0.15	24.71±2.56	3.96±0.13	81.33±3.83	2.89±0.14	
Furans (1)	2-pentyl furan	3777-69-3	1229	1249	nd.	nd.	1.75±0.11 b	nd.	0.54±0.08 c	nd.	nd.	4.45±1.29 a	butter, floral, fruit, green bean

“RI cal” means RI calculated by the retention time of alkanes. “RI lit” means RI listed in the literature. Compounds with“*” means variables with VIP score > 1. “nd.” means not detected.

PLS-DA and bi-plot (Figure 3-6; Figure 3-7) got from Table 3-4 showed the same tendency as the PCA results obtained from GC-IMS and E-nose (Figure 3-3 and Figure 3-5). SNXDL, SDZH, HBHH, SDYT, XJKEL, and SXLY were relatively clustered, while SXYC and XJAKS were apart from the others. As shown in Figure 3-8, 1-peten-3-ol, acetone, 2-methoxyphenol, methyl laurate, 3-pentanone, 2-formyltoluene and ethyl hexanoate were the components with VIP scores > 1, indicating that they were crucial components for the discrimination of winter jujubes from eight regions. The relationship between the differential VOCs (Table 3-4) and sensors is visualized by heatmap (Figure 3-9). The potential markers for SXYC and XJAKS were analyzed according to the VIP scores and heatmap.

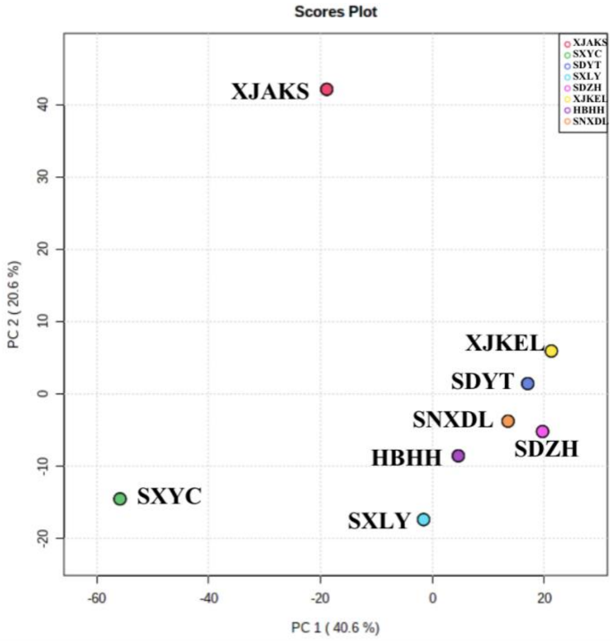


Figure 3-6 Partial least squares discrimination analysis (PLS-DA) analysis of the different cultivars of winter jujubes based on differential compounds

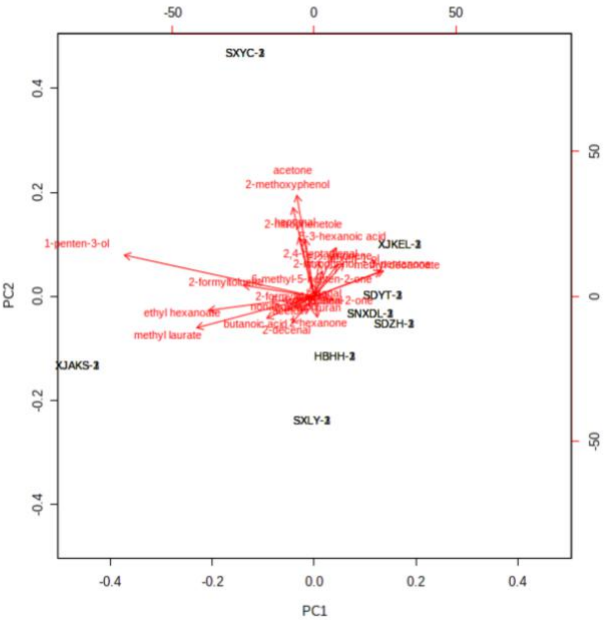


Figure 3-7 Bi-plot analysis of the differential compounds in winter jujubes

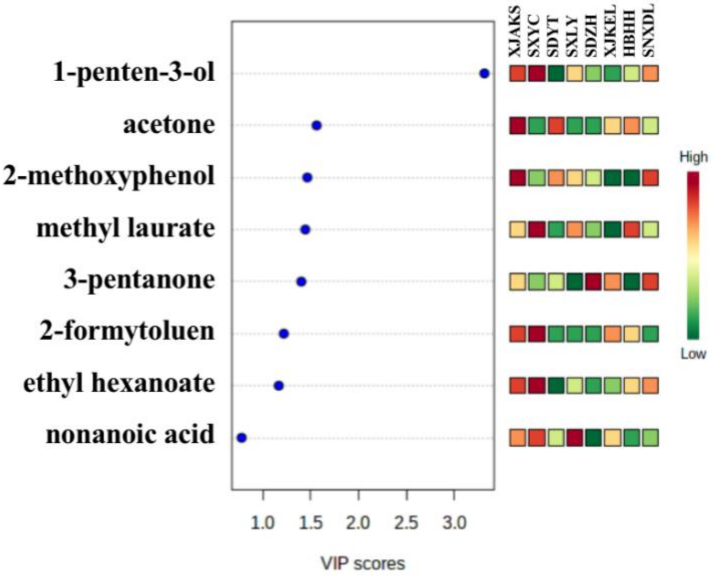


Figure 3-8 VIP scores of the differential compounds in winter jujubes

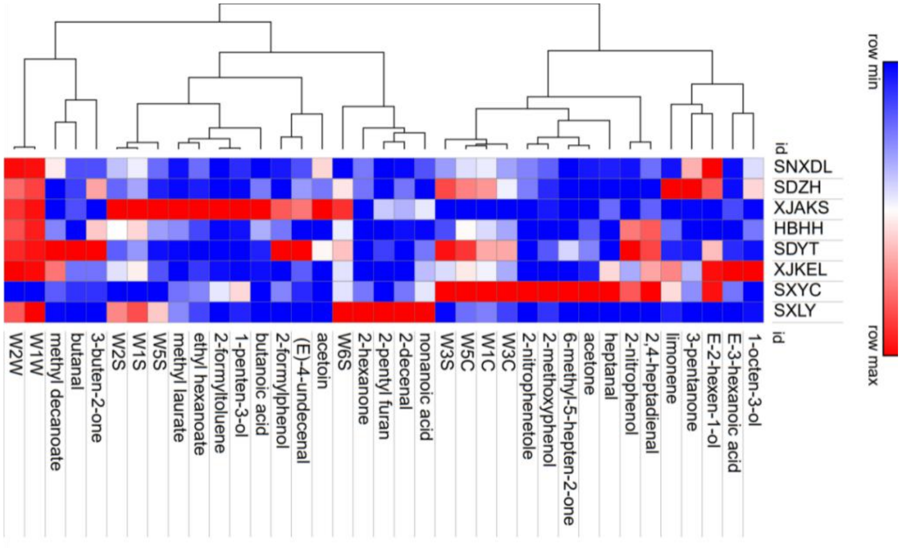


Figure 3-9 Correlation analysis between different components and sensors by heatmap

For SXYC, the sensors with higher response values (W1C, W3C and W5C) were closely related with heptanal, acetone, 6-methyl-5-hepten-2-one, 2-methoxyphenol and 2 -nitrophenetole (Figure 3-8). However, only acetone and 2-methoxyphenol had VIP scores over 1. Research showed 2-methoxyphenol was a product of pyrolysis of lignin with woody odor. Acetone existed naturally in plants with pungent odor (Ishida et al., 2014). The quantitative results in GC-MS showed that 2-methoxyphenol and acetone accounted for 31.67% of the differential VOCs in SXYC (Table 3-2). Their concentration in SXYC was several times higher than that in other jujubes (Table 3-2). Hence, 2-methoxyphenol and acetone might be the potential markers of SXYC with a woody and pungent aroma.

For XJAKS, the sensors with higher response values (W5S, W1S and W2S) were in close relationship with acetoin, (E)-4-undecenal, 2-formylphenol, butanoic acid, 1-penten-3-ol, 2-formyltoluene, ethyl hexanoate and methyl laurate (Figure 3-8). However, 1-penten-3-ol, ethyl hexanoate, methyl laurate and 2-formyltoluene were the ones with VIP scores > 1, and their amount comprised 74.65% of the different VOCs in XJAKS. 1-penten-3-ol was one of the secondary lipid oxidation products. It was once found in oolong tea infusions and it was responsible for butter and pungent odor (Zhu et al., 2015). Ethyl compounds like ethyl hexanoate exist in fruits (Shi et al., 2020). Methyl laurate could effectively inhibit enzyme activities and thus could help to prevent the green color of fruits from fading away (Yamauchi et al., 2008). Methylbenzaldehydes were present in tomato, cider, elderberry juice, tea, etc. (Miyazawa et al., 2011). 2-Formyltoluene was found in winter jujube for the first time.

Thus 1-penten-3-ol, ethyl hexanoate, methyl laurate and 2-formyltoluene might be the potential markers for XJAKS with green and fruity-like aroma (Figure 3-10).

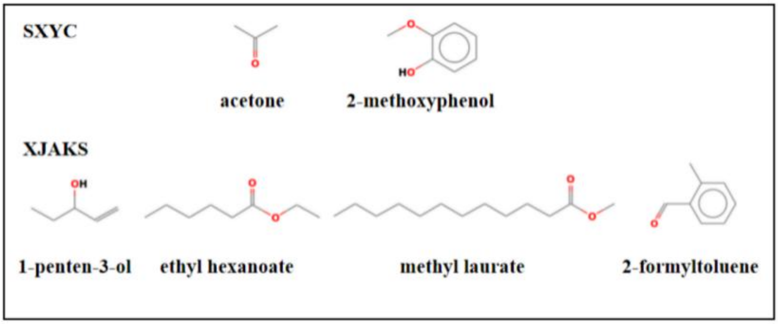


Figure 3-10 Potential markers in SXYC and XJAKS

4. Conclusion

In conclusion, the difference and similarity in VOCs of winter jujube from eight regions of China were well analyzed by GC-IMS, E-nose, and GC-MS. All results showed that SNXDL, SDZH, HBHH, SDYT, XJKEL and SXLY clustered together with the differentiation of SXYC and XJAKS. Benzyl alcohol, octanoic acid, 2-hexenal, linalool, 2-nonenal and ethyl decanoate were the common volatiles of winter jujubes from eight regions of China. The combined analysis of VIP scores, heatmap and aroma description indicated 1-penten-3-ol, ethyl hexanoate, methyl laurate and 2-formyltoluene were the potential markers for green and fruity aroma profile of SXYC, XJAKS could be labeled by acetone and 2-methoxyphenol with wood-like and pungent aroma.

4

Chapter Ⅳ. Investigation of the volatile profile of red jujube by using GC-IMS, multivariate data analysis, and descriptive sensory analysis

Aroma characteristics of six red jujube cultivars (Jinchang- 'JC', Junzao- 'JZ', Huizao- 'HZ',Qiyuexian- 'QYX', Hetiandazao- 'HTDZ', and Yuanzao- 'YZ') cultivated in Xinjiang province, China, were studied by E-nose and GC-IMS in Chapter 4. Several studies on phytochemical profiling, bioactive components, pathogenic factor analysis have been carried out to better understand the quality gained by red jujube cultivars planted in Xinjiang. The aim of this study was to investigate the aroma profile of red jujubes cultivars from Xinjiang Province, China.

Qiao, Y., Chen, Q., Bi, J., Wu, X., Jin, X., Gou, M., ... & Purcaro, G. (2022). Investigation of the Volatile Profile of Red Jujube by Using GC-IMS, Multivariate Data Analysis, and Descriptive Sensory Analysis. *Foods*, 11(3), 421.

Abstract

Aroma characteristics of six red jujube cultivars (Jinchang- ‘JC’, Junzao- ‘JZ’, Huizao- ‘HZ’,Qiyuexian- ‘QYX’, Hetiandazao- ‘HTDZ’, and Yuanzao- ‘YZ’) cultivated in Xinjiang province, China, were studied by E-nose and GC-IMS. The presence of acetoin, E-2-hexanol, hexanal, acetic acid, and ethyl acetate played an important role in the classification results. JC, JZ, HZ and YZ were different from others, while QYX and HTDZ were similar to each other. HZ had the most abundant specific VOCs, including linalool, nonanoic acid, methyl myristoleate, 2-acetylfuran, 1-octen-3-one, E-2-heptenal, 2-heptenone, 7-octenoic acid, and 2-pentanone. HZ had higher intensity in jujube ID (identity), floral, sweet, and fruity attributes. Correlation analysis showed jujube ID might be related to phenylacetaldehyde and isobutanoic acid that formed by the transamination or dehydrogenation of amino acids, meanwhile sweet attribute was correlated with amino acids including threonine, glutamic acid, glycine, alanine, valine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine.

Keywords: Red jujube, Aroma distinction, E-nose, GC-IMS, Sensory attributes

1. Introduction

Jujube (*Ziziphus Jujuba* Mill.), belonging to the family Rhamnaceae, is a plant largely distributed in tropical and sub-tropical regions. In particular, it is widely distributed in China, where its fruits are regularly consumed for their good aroma, delicious taste, and high nutraceutical value in the Chinese medicine tradition. Moreover, at present, red jujubes are also widely used in food industry (the ingredient of tea, snack, bread, cakes, yogurt, etc.) (Rashwan et al., 2020). There are over 1000 varieties of red jujube cultivated in China with distribution in Xinjiang, Gansu, Ningxia, Shaanxi, Shanxi, Shandong, Hebei, and Henan Provinces (Ji et al., 2017), but Xinjiang Province (latitude: 34°22′N ~ 49°10′N; longtitude: 73°40′E ~ 96 °23′E; elevation: 967.2 m ~ 1388.78 m) is by far the largest jujube production region in China with an annual output of more than 1.45 million tons in 2018 (Wang et al., 2020). The quality of red jujubes from Xinjiang is superior to other regions because of the low rainfall with periodic drought, abundant sunshine, and substantial differences between day and night temperatures (Wang et al., 2019). Different cultivars of red jujubes are present in China, among which Huizao (HZ), Junzao (JZ), Yuanzao (YZ), Qiyuexian (QYX), Jinchang (JC), and Hetiandazao (HTDZ) are the most widely cultivated in different regions of Xinjiang Province. Huizao (HZ) alone accounts for 62.9% of the production from the region, followed by Junzao (JZ) which accounts for 32.7%. Moreover, Charkhlik HZ and Khotan JZ are two cultivars of protected geographical indication (Wang et al., 2020). Several studies on phytochemical profiling, bioactive components, and pathogenic factor analysis have been carried out to understand better the quality gained by red jujube cultivars planted in Xinjiang (Zhang et al., 2018; Song et al., 2019). Nevertheless, no studies have been done to investigate the particular aroma profile of the red jujube cultivated in Xinjiang Province and correlate the sensory characteristics with a chemical composition including VOCs, fatty acids, amino acids, organic acids, and sugars.

In fact, the primary metabolite distribution in fruits (including sugars, organic acids, and amino acids) may be associated with sensory traits like sweetness and sourness, both as precursors of VOCs related to aroma and taste or as involved in the browning reaction (Tieman et al., 2006; Tran et al., 2012). For example, the content of sucrose in baked potatoes had an influence on the sweetness (Chan et al., 2014). The composition of citric, quinic, and malic acid had a strong relationship with sourness in blackcurrant juice (Laaksonen et al., 2012). Threonine, serine, and alanine were found to be correlated with the sweet attribute, aspartate and glutamate were correlated with the sour attribute, and valine, methionine, isoleucine, leucine, and arginine were found to contribute to bitter taste in table grape berries (Pinsorn et al., 2018).

E-nose has been applied to discriminate the aroma quality of food successfully, such as the varieties of jujube and *Lycium ruthenicum* Murray from different provinces, harvest years, and varieties (Rodríguez et al., 2014; Kovacs et al., 2010; Zhang & Pawliszyn, 1993; Chen et al., 2018; Liu et al., 2021; Wang et al., 2019). The sum of PC1 and PC2 was between the range of 80 %~98% for the classifications. The aim of this work is to investigate the aroma profile of red jujube cultivars cultivated in Xinjiang Province by using GC-IMS and E-Nose and investigate the relationship between the sensory perception and the characteristic VOCs and metabolite precursors.

The data from the volatile profile was elaborated at different levels: i) to discriminate among the different cultivars of red jujube (JC, JZ, HZ, QYX, HTDZ, and YZ cultivated in Xinjiang Province; ii) to investigate the correlation between sensory attributes and chemical compositions including VOCs, fatty acids, amino acids, organic acids, and sugars.

2. Materials and methods

2.1. Plant material preparation

Six cultivars of red jujubes (*Ziziphus Jujuba* Mill., JC, JZ, HZ, QYX, HTDZ, and YZ, each cultivar with three biological repeats) which grown in Xinjiang Province of China were used in this study (Table 4-1). The botanical identification was confirmed by expert botanists. Fifty kg of each cultivar of red jujube were bought at a mature commercial stage in the Beiyuanchun Jujube Market (Urumqi, Xinjiang Province, China, 2018). Red jujubes without physical injuries and/or infections were selected and stored at 4°C before analysis within 72h.

Table 4-1 Information about six cultivars of red jujube in Xinjiang Province, China

Code	Variety	Origin	Traits
QYX	Qiyuexian	Xinjiang, Akesu	
JC	Jinchang	Xinjiang, Akesu	
HTDZ	Hetiandazao	Xinjiang, Khotan	
YZ	Yuanzao	Xinjiang, Hami	
HZ	Huizao	Xinjiang, Charkhlik	
JZ	Junzao	Xinjiang, Khotan	

2.2. Chemicals and regents

The chemical standards of formic acid, hexanoic acid, propionic acid, acetic acid, isobutyric acid, 3-methylbutanoic acid, pentanoic acid, 3-heptenoic acid, nonanoic acid, crotonic acid, 7-octenoic acid, n-decanoic acid, heptanoic acid, 2-heptenoic acid, ethanol, linalool, E-2-hexenol, 1-octen-3-ol, 5-methyl-2-furanmethanol, 6-methyl-5-hepten-2-ol, 1-nonen-4-ol, butanal, 2-methylbutanal, 3-methylbutanal, hexanal, furfurol, benzaldehyde, E-2-heptenal, E-2-octenal, n-nonanal, phenylacetaldehyde, pentanal, acetoin, 6-methyl-5-hepten-2-one, 1-octen-3-one, 2-pentanone, acetone, 3-octanone, 2-hexanone, 2-heptanone, methyl acetate, ethyl acetate, ethyl propanoate, propyl acetate, ethyl 2-hydroxypropanoate, ethyl 3-methylbutyrate, butyl acetate, isoamyl acetate, ethyl pentanoate, butyrolactone, ethyl hexanoate, methyl myristoleate, methyl hexanoate, ethyl benzoate, methyl benzoate, ethyl heptanoate, hexyl butanoate, gamma-terpinene, alpha-phellandrene, myrcene, limonene, 2-acetylfuran, 2-pentyl furan, o-cymene, and 2-cyclohexenone were purchased from MilliporeSigma (St. Louis, MO, USA).

Malic acid, citric acid, quinic acid, lactic acid, tartaric acid, glucose, fructose, maltose, sodium hydroxide, and potassium hydroxide were of HPLC grade (Sinopharm Chemical Reagent Co., Ltd., Beijing, China). Glutamic acid (GLU) and aspartic acid (ASP) were obtained from MilliporeSigma; serine (SER), threonine (THR), aspartic acid (ASP), glycine (GLY), alanine (ALA), citrulline (CIT), Valine (VAL), methionine (MET), isoleucine (ILE), leucine (LEU), tyrosine (TYR), lysine (LYS), histidine (HIS), arginine (ARG), and proline (PRO) were purchased from Wako Pure Chemical Industries, Ltd (Chuo-ku, Japan).

2.3. Sample preparation

The detailed procedure of sample preparation was referred to Chen et al. (2018). About 100 g of red jujubes were washed with running water and the moisture on the surface was wiped with filter papers, kernel removed, sliced and ground for 90 s with a juicer (JYL-CO20, Joyoung Co., Ltd., Shandong, China). A weight of 2.0 g red jujube pulp was put into a 20 mL vial that was sealed with a magnetic screw-cap and septum before E-nose and GC-IMS testing.

2.4. E-Nose analysis

A commercial PEN 3.5 E-Nose (Airsense Analytics, GmBH, Schwerin, Germany) containing ten metal-oxide semiconductors (MOS) (Table 4-2) was used to distinguish among the overall aroma perception of the six cultivars of red jujubes. The samples were equilibrated at room temperature (26 ° C) for 30 minutes. The gaseous compounds in the headspace were firstly pumped through a Teflon tube into the sensor arrays at 400mL/min using reference air (filtered through charcoal). This clean air was also used for the rinse of the system at a flow rate of 600 mL/min. The cleaning time, zero adjustment time, and detection time were 180s, 10s, and 60s, respectively. During the monitoring of the sample gas, the ratio of G (the conductance of a sensor exposed to sample gas) to G0 (the conductance of a sensor exposed to zero gas) for each sensor would be changed, which could be processed by WinMuster Software and reflecting the information of the sample gas's composition. Five repeats from the same 100g sample were prepared.

Table 4-2 Response characteristics and repeatability of response values of ten sensors in E-nose

Number	Sensors	Response characteristics	RSD/%
1	W1C	aromatic compounds	0.18
2	W5S	nitroxide	4.54
3	W3C	ammonia and aromatic components	0.28
4	W6S	hydrogen selective	0.85
5	W5C	alkanes and aromatic components	1.65
6	W1S	methane	0.81
7	W1W	sulfides	0.65
8	W2S	ethyl alcohol	0.74
9	W2W	aromatic components and organic sulfide	0.59
10	W3S	alkanes	0.56

Note: 'RSD' is the abbreviation of relative standard deviation.

2.5. HS-GC-IMS analysis

A GC-IMS (GC from Agilent Technologies, Palo Alto, CA, USA; IMS from FlavourSpec®, Gesellschaft für Analytische Sensorsysteme mbH, Dortmund, Germany) system equipped with an autosampler unit (CTC Analytics AG, Zwingen, Switzerland) was applied in this project. The vials prepared as in 2.3 were incubated at 50°C for 20 min. Then a headspace volume of 500 μL was collected from the vials with a heated syringe (85 ° C) and injected at 80 ° C in splitless mode. GC conditions: the chromatographic column was a FS-SE-54-CB-1 (15m×0.53mm ID× 1.0 μm df) column (60 ° C isothermal conditions). The program for carrier gas (nitrogen, 99.999%) was as follows: 2 mL/min for 2 min, ramped up to 10 mL/min over 8 min, then reached 100 mL/min over 10 min, at last got to 150 mL/min with 5 min. All the standards presented in section 2.1 were run under the same test procedure to support the GC-IMS Library Search for qualitative analysis. For the semi-quantitative analysis, 2-cyclohexenone (2 mg/L) was selected as the internal standard.

IMS conditions: ion source was a tritium source (5.68 keV), positive ion mode, drift tube length 9.8 cm, tube linear voltage 500 V/cm, drift gas flow rate 150 mL/min (nitrogen, purity 99.999%). The temperature of the drift tube was 45 °C.

The spectrogram was elaborated using Laboratory Analytical Viewer (LAV). The Reporter Plug-In software was used to compare spectrogram differences between samples. Gallery Plot Plug-In was used to compare the differences between volatile fingerprints visually and quantitatively. Three technical repeats were prepared for each cultivar.

2.6. Sensory analysis

The sensory analysis was conducted according to the method set by Galindo et al. (Galindo et al., 2015). The sensory analysis aimed to evaluate the following sensory attributes: sour, sweet, bitter, astringent, jujube ID, fruity and floral (Galindo et al., 2015). The intensities of the various sensory attributes were evaluated using a numerical scale where 0 represents none and 10 extremely strong, with 0.1 increments. A panel of twenty trained assessors (aged 21-30, ten females and ten males) was invited to evaluate the flavor of red jujubes. The assessors were recruited from fruit and vegetable processing teams and had extensive experience in fruit sensory evaluation. For the evaluation, fifteen red jujubes for each cultivar per panelist were served in the testing room at 26 °C. There was a 10 min wait among the sensory evaluation for different cultivars.

2.7. Analysis of fatty acids, amino acids, monosaccharides, and organic acids

The determination of fatty acids was referred to the method reported by Song et al. (2019) using a GC - flame ionization detector (FID) (GC6890N, Agilent, USA) (Song et al., 2019). Undecanoic acid (5 mg/mL) was used as an internal standard and the results were expressed on mg/g dry basis. Analyses were carried out in triplicate.

The determination of free amino acids was referred to Song et al. (Song et al., 2019). Using an Hitachi model L-8900 amino acid analyzer (Hitachi Co. Ltd., Tokyo, Japan) with a column packed with Hitachi custom ion-exchange resin 2622 (4.6 mm × 60 mm, particle size 5 μm).

Quantitative analysis of monosaccharides and organic acids in red jujubes was performed by high-performance anion-exchange chromatography equipped with pulsed amperometric detection (Dionex ICS-3000 system) according to Šimkovic et al. (Šimkovic et al., 2009).

The standards of sugars and organic acids were tested at the same condition quantitative analysis using external calibrations.

2.8. Statistical analysis

All the experiments were carried out in triplicate, and data was expressed as mean ± SD. Statistic difference among diversely treated samples was calculated through one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. The IBM SPSS 24.0 (Chicago, USA) software was used, and statistical difference was considered significant when $p < 0.05$.

3. Results and discussion

3.1. E-nose analysis

The averaged response values of different red jujube samples on ten MOS sensors are reported in Figure 4-1. The response values showed a relative standard deviation (RSD) lower than 5% (Table S2). Response signals for different red jujube samples increased at different rates. The most significant increase and the highest level in response signals were found in W1W sensitive to sulfides (minimum and maximum were 10.5~31.9), followed by W5S sensitive to nitroxide (6.0~29.0), W2W sensitive to aromatic components and organic sulfide (4.6~12.4), W2S (2.8~5.0) and W1S sensitive to methane (2.6~4.7). However, few responses were shown in the sensors, including W6S, W3C, W5C, W3S, and W1C (around 1.0), sensitive to hydrogen, ammonia and aromatic components, alkanes and aromatics, alkanes, and aromatics, respectively. The response values of W1W (31.9), W5S (29.0), and W2W (12.4) were especially higher in HZ, followed by JZ (W1W, 20.2; W5S, 15.6; W2W, 8.1), JC (W1W, 17.5; W5S, 10.1; W2W, 7.1) and YZ (W1W, 12.2; W5S, 8.1; W2W, 5.1). As shown in Figure 1, QYX and HTDZ showed the same response level in the sensors of W1W, W2W, W2S, W1S , W3S, and W5C, compared with other cultivars.

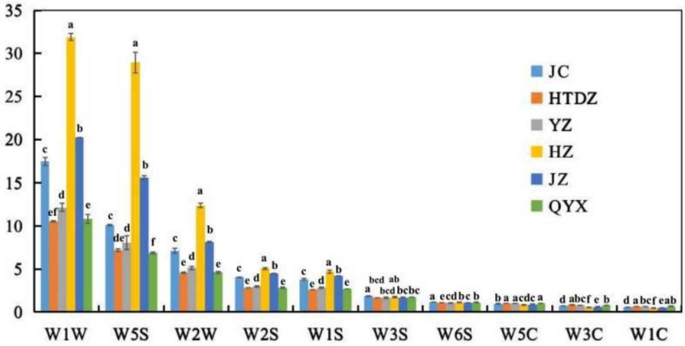


Figure 4-1 The response data of sensors in E-nose on volatiles of the six red jujube cultivars in Xinjiang province, China (Jinchang-‘JC’, Junzao-‘JZ’, Huizao-‘HZ’,Qiyuexian-‘QYX’, Hetiandazao-‘HTDZ’, and Yuanzao-‘YZ’).

The data were processed using a PCA to visualize whether the E-nose measurement was able to distinguish among the different red jujube cultivars. The sum of PC 1 and PC 2 account for 71.7% of the E-nose results (Figure 4-2). The technical repeats for the six cultivars were well clustered, except for a slight overlap of the HTDZ and QYX cultivars. The response value on W1W played an important role in the clustering of HTDZ and QYX (Figure 4-2). Meanwhile, the separation of JZ was mainly caused by the response value of W1C.

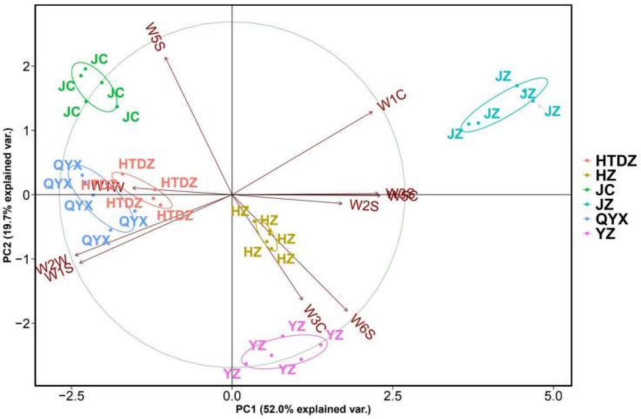


Figure 4-2 Principle component analysis (PCA) and loading plot of of E- nose data for the six red jujube cultivars in Xinjiang province, China (Jinchang-‘JC’, Junzao-‘JZ’, Huizao-‘HZ’,Qiyuexian-‘QYX’, Hetiandazao-‘HTDZ’, and Yuanzao-‘YZ’).

E-nose cannot provide qualitative information on the specific chemical compounds responsible for the observed discrimination, but when properly trained and validated with more information-rich techniques (e.g. GC-IMS), it could be used a valid routine method to discriminate the different jujube varieties. Anyway, it is a valid routine method when the chemical basis is well-understood. Therefore, additional qualitative and quantitative characterization of the volatiles was performed by GC-IMS.

3.2. GC-IMS analysis

The chromatograms of GC-IMS are shown in Figure 4-3. Sixty-four compounds were identified by GC-IMS (Table 4-3) in the six cultivars of red jujubes including esters (17), acids (14), aldehydes (11), ketones (8), alcohols (6), terpenoids (6), and furans (2). Sixteen compounds including hexanoic acid, isobutyric acid, 3-methylbutanoic acid, n-decanoic acid, heptanoic acid, 1-octen-3-ol, hexanal, benzaldehyde, E-2-heptenal, n-nonanal, 6-methyl-5-hepten-2-one, ethyl hexanoate, ethyl benzoate, methyl benzoate, 2-pentyl furan, and o-cymene) had been reported in previous studies (Chen et al., 2018).

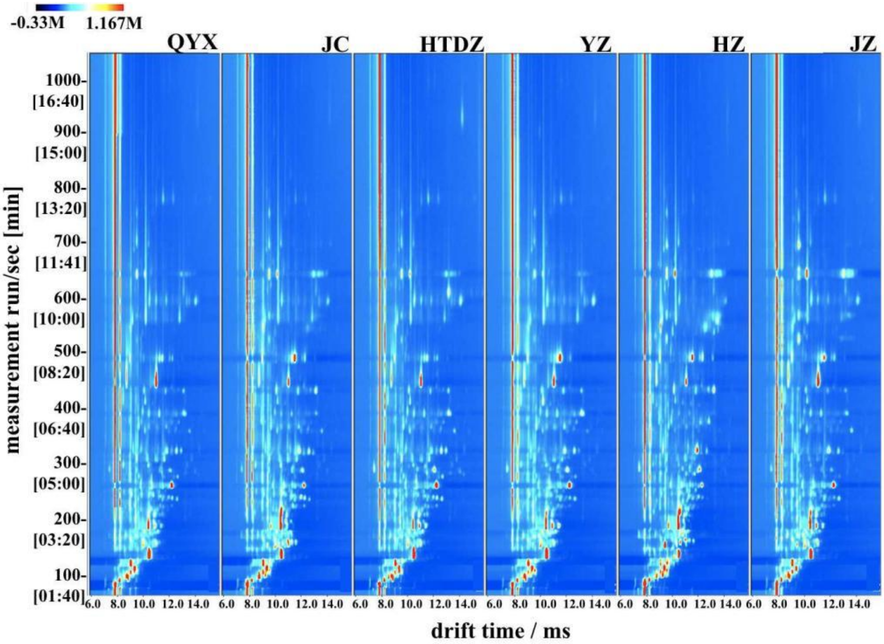


Figure 4-3 The combined chromatograms of the six red jujube cultivars in Xinjiang province, China (Jinchang-'JC', Junzao-'JZ', Huizao-'HZ',Qiyuexian-'QYX', Hetiandazao-'HTDZ', and Yuanzao-'YZ').

Table 4-3 The GC-IMS results of the volatile composition of the six red jujube cultivars.

	Compound	RI	Rt [sec]	Dt [a.u.]	Concentration(μg/g)					
					Qiyuexian (QYX)	Jinchang (JC)	Hetianda zao (HTDZ)	Yuanzao (YZ)	Huizao (HZ)	Junzao (JZ)
Acids（14）	formic acid	568.30	130.64	1.16	0.30±0.08c	0.27±0.07a	0.32±0.03a	0.37±0.07b	0.26±0.14a	0.40±0.05d
	hexanoic acid	576.70	134.37	1.18	0.26±0.02ab	1.01±0.04a	0.26±0.08ab	0.40±0.04b	1.47±0.05c	0.42±0.11b
	propionic acid	659.50	170.83	1.44	0.05±0.02bc	0.08±0.01cd	0.06±0.01b	0.07±0.02a	0.18±0.02d	0.05±0.03a
	acetic acid	659.90	170.99	1.48	0.04±0.01d	0.06±0.04b	0.04±0.01d	0.06±0.04c	0.07±0.01a	0.04±0.01c
	isobutyric acid	749.20	234.36	1.56	0.58±0.02ab	1.26±0.02b	0.55±0.02a	0.73±0.01b	2.52±0.02c	0.83±0.01c
	3-methyl butanoic acid	749.20	234.36	1.45	0.14±0.02ab	0.37±0.05a	0.13±0.02a	0.22±0.04c	0.62±0.02b	0.27±0.01c
	pentanoic acid	844.30	333.33	1.61	2.16±0.03bc	1.30±0.03ab	2.83±0.05a	2.26±0.05a	0.96±0.03c	1.73±0.04d
	3-heptenoic acid	1055.60	692.06	1.53	3.22±0.02d	9.07±0.01c	3.26±0.01c	4.62±0.02d	10.95±0.02b	4.68±0.04a
	crotonic acid	807.30	290.37	1.41	1.91±0.01c	2.82±0.01b	1.90±0.02c	2.03±0.02b	1.84±0.01a	1.92±0.04c
	nonanoic acid	1024.20	632.73	1.39	0.21±0.01b	0.54±0.01a	0.74±0.01b	0.65±0.03a	1.24±0.02a	0.15±0.02b
	7-octenoic acid	905.90	414.10	1.23	0.04±0.01c	0.08±0.00bc	0.05±0.01bc	0.09±0.01b	0.16±0.01a	0.06±0.01d
	n-decanoic acid	887.30	383.31	1.56	0.05±0.01b	0.06±0.01a	0.08±0.01b	0.07±0.02b	0.07±0.01a	0.06±0.03b

	heptanoic acid	1219.70	1002.43	1.54	0.96±0.02b	1.19±0.01a	1.32±0.04b	1.62±0.05b	0.97±0.02a	1.20±0.01b
	2-heptenoic acid	1012.90	611.23	2.11	0.10±0.01e	0.25±0.01b	0.17±0.01d	0.08±0.02c	0.46±0.04a	0.41±0.01e
	Total				10.02±0.29f	18.36±0.32c	11.71±0.33e	13.27±0.44d	21.77±0.42b	22.22±0.42a
Alcohols (6)	ethanol	532.50	114.88	1.16	2.31±0.14b	3.15±0.04ab	3.00±0.03a	2.53±0.02ab	3.06±0.17a	1.71±0.09b
	E-2-hexenol	730.10	218.38	1.47	0.14±0.01e	0.44±0.02b	0.18±0.01e	0.17±0.04d	0.83±0.07a	0.25±0.03c
	1-octen-3-ol	771.00	252.57	1.26	0.09±0.02c	0.18±0.04b	0.11±0.03c	0.08±0.02c	0.26±0.02a	0.12±0.01c
	5-methyl-2-furanmethanol	1041.70	665.70	1.14	0.77±0.01e	1.14±0.02b	0.66±0.01d	0.63±0.01de	0.78±0.04a	0.80±0.01c
	6-methyl-5-hepten-2-ol	650.60	166.89	1.28	0.19±0.00e	0.1±0.01b	0.28±0.00e	0.15±0.01d	0.07±0.02a	0.09±0.03c
	1-nonen-4-ol	812.20	296.10	1.79	0.16±0.00cd	0.19±0.03b	0.22±0.02c	0.15±0.02d	0.11±0.00a	0.24±0.05d
	Total				3.66±0.18bc	5.20±0.16a	4.45±0.97ab	3.71±0.12bc	5.11±0.54a	3.21±0.22d
Aldehydes (11)	butanal	563.50	128.57	1.22	0.54±0.01cd	0.52±0.01b	0.57±0.02c	0.52±0.02c	0.42±0.05a	0.45±0.02d
	2-methylbutanal	642.20	163.20	1.20	0.53±0.00b	0.59±0.00a	0.61±0.01a	0.62±0.02b	0.51±0.03d	0.30±0.01c
	3-methylbutanal	652.10	167.55	1.35	0.10±0.02cd	0.77±0.01a	0.15±0.04c	0.09±0.06d	0.48±0.02b	0.65±0.04d
	hexanal	742.10	228.36	1.26	0.19±0.01c	0.37±0.01e	0.25±0.07a	0.32±0.07b	0.42±0.01f	0.17±0.07d
	furfurol	735.80	223.09	1.09	0.19±0.00b	0.38±0.02a	0.22±0.01bc	0.21±0.01c	0.72±0.01d	0.15±0.01e
	benzaldehyde	815.40	299.81	0.95	0.03±0.03e	0.06±0.08b	0.04±0.03e	0.03±0.04c	0.17±0.01a	0.04±0.05d

	E-2-heptenal	973.50	538.12	1.11	0.08±0.01c	0.07±0.01b	0.10±0.03c	0.12±0.01c	0.44±0.01a	0.06±0.01c
	E-2-octenal	645.60	164.68	1.31	5.38±0.01de	6.60±0.01b	6.71±0.01cd	6.70±0.01e	4.46±0.01a	5.12±0.01c
	n-nonanal	890.40	386.83	1.11	0.41±0.02b	0.58±0.03e	0.44±0.01a	0.62±0.02d	0.55±0.01f	0.43±0.01c
	phenylacetaldehyde	729.40	217.77	1.44	0.46±0.01c	0.54±0.01a	0.71±0.01bc	0.70±0.02c	0.46±0.01ab	0.52±0.03c
	pentanal	1181.60	930.25	1.82	0.02±0.02d	0.03±0.01bc	0.03±0.01cd	0.03±0.02a	0.07±0.01ab	0.03±0.01e
	Total				7.93±0.14e	10.51±0.20a	9.83±0.25bc	9.96±0.30b	8.70±0.18d	7.92±0.27e
Ketones (8)	acetoin	705.00	197.35	1.23	0.08±0.02d	0.09±0.01b	0.08±0.01d	0.10±0.04c	0.24±0.03a	0.06±0.01c
	6-methyl-5-hepten-2-one	772.50	253.78	1.34	0.29±0.01c	0.18±0.02b	0.31±0.01b	0.23±0.02c	0.15±0.01a	0.26±0.02d
	1-octen-3-one	1059.50	699.44	1.22	0.03±0.03d	0.05±0.02b	0.04±0.01d	0.06±0.02e	0.08±0.01a	0.04±0.01c
	2-pentanone	1022.70	629.87	1.23	0.29±0.01d	0.66±0.01c	0.39±0.01c	0.46±0.01b	0.84±0.01a	0.25±0.01d
	acetone	1016.50	618.22	1.90	0.76±0.02bc	1.16±0.07a	0.97±0.00bc	0.65±0.06b	1.04±0.20c	0.94±0.02bc
	3-octanone	1017.80	620.55	1.20	0.03±0.03b	0.05±0.04b	0.03±0.02b	0.02±0.04b	0.08±0.05a	0.06±0.03c
	2-hexanone	921.30	442.41	1.50	0.26±0.01d	0.31±0.02b	0.29±0.03c	0.15±0.01b	0.53±0.03a	0.82±0.02cd
	2-heptanone	1015.20	615.62	1.68	0.40±0.01d	0.18±0.03de	0.64±0.01c	0.76±0.01b	1.00±0.01a	0.24±0.04e
	Total				2.14±0.14bc	2.68±0.42abc	2.75±0.46ab	2.43±0.21bcd	3.96±0.89a	2.67±0.16bc
Esters (17)	methyl acetate	545.70	120.69	1.19	2.10±0.03bc	2.20±0.04ab	2.15±0.03cd	2.07±0.04d	1.33±0.12b	2.04±0.01b

ethyl acetate	583.80	137.48	1.24	0.58±0.09b	0.60±0.06a	0.63±0.06a	0.39±0.06a	0.53±0.30c	0.33±0.03c
ethyl propanoate	733.10	220.87	1.39	0.04±0.01d	0.11±0.01e	0.06±0.02b	0.05±0.02a	0.16±0.01f	0.05±0.02c
propyl acetate	767.10	249.34	1.23	1.46±0.02d	2.42±0.06a	1.67±0.01b	1.42±0.01c	1.26±0.01b	1.46±0.05d
ethyl 2-hydroxypropanoate	767.70	249.84	1.54	0.35±0.03e	0.89±0.02d	0.37±0.05b	0.36±0.07c	1.20±0.01a	0.32±0.01e
ethyl 3-methylbutyrate	746.10	231.70	1.48	0.03±0.01b	0.04±0.01d	0.03±0.01a	0.03±0.01c	0.05±0.01e	0.03±0.00de
butyl acetate	759.40	242.88	1.12	0.54±0.03b	0.63±0.03c	0.61±0.01a	0.52±0.01b	0.40±0.02d	0.46±0.03c
isoamyl acetate	779.20	259.43	1.08	0.85±0.05d	2.00±0.01c	0.89±0.01b	0.82±0.04d	1.22±0.03e	0.81±0.01a
ethyl pentanoate	811.90	295.77	1.46	0.28±0.02c	0.30±0.00e	0.27±0.02b	0.25±0.02a	0.16±0.01f	0.12±0.01d
butyrolactone	815.40	299.81	1.37	0.58±0.04b	1.14±0.06a	0.62±0.01b	0.90±0.01a	1.24±0.03a	0.72±0.02b
ethyl hexanoate	899.10	401.57	1.34	0.02±0.02c	0.04±0.03b	0.03±0.02a	0.02±0.04a	0.03±0.06c	0.02±0.05bc
methyl myristoleate	881.20	376.19	1.41	0.10±0.01c	0.14±0.01b	0.13±0.01c	0.27±0.02c	0.58±0.01a	0.09±0.00c
methyl hexanoate	826.10	312.30	1.26	0.29±0.01e	0.20±0.02b	0.60±0.01d	0.79±0.03c	0.14±0.01a	0.40±0.01f
ethyl benzoate	880.50	375.38	1.33	0.05±0.01b	0.11±0.01a	0.09±0.01b	0.07±0.01a	0.09±0.01b	0.06±0.01b
methyl benzoate	759.30	242.76	1.38	0.01±0.00c	0.12±0.01a	0.01±0.00c	0.01±0.02c	0.10±0.04b	0.01±0.01bc
ethyl heptanoate	766.60	248.85	1.43	0.15±0.02b	0.25±0.01b	0.17±0.01a	0.15±0.01b	0.21±0.01b	0.17±0.01b
hexyl butanoate	765.20	247.72	1.47	0.08±0.01c	0.08±0.01bc	0.12±0.06a	0.09±0.01b	0.07±0.03ab	0.07±0.01bc

	Total				7.51±0.41e	11.27±0.40a	8.45±0.34bc	8.21±0.43bcd	8.77±0.72b	7.16±0.29ef
Terpenoids (6)	gamma-terpinene	693.40	187.66	1.29	0.04±0.03d	0.05±0.05c	0.05±0.03b	0.04±0.05a	0.06±0.02d	0.05±0.07c
	alpha-phellandrene	1055.90	692.69	1.19	0.18±0.02e	0.22±0.01c	0.20±0.01d	0.26±0.01f	0.24±0.02a	0.09±0.01b
	myrcene	909.60	420.86	1.37	0.06±0.02de	0.10±0.01c	0.06±0.01cd	0.04±0.00b	0.25±0.03a	0.09±0.01f
	limonene	810.50	294.08	1.69	0.11±0.02d	0.08±0.06b	0.15±0.02c	0.12±0.01e	0.06±0.02a	0.08±0.02c
	linalool	766.50	248.84	1.56	0.04±0.01d	0.08±0.01b	0.05±0.01d	0.06±0.01c	0.16±0.01a	0.05±0.01c
	o-cymene	783.00	262.64	1.41	1.11±0.02c	1.15±0.03c	1.09±0.04c	1.15±0.02d	1.33±0.02b	2.76±0.02a
	Total				1.54±0.12de	1.68±0.17c	1.60±0.12cd	1.67±0.10c	2.10±0.12b	3.12±0.14a
Furans (2)	2-acetylfuran	970.60	532.87	1.22	0.29±0.01b	0.36±0.02b	0.37±0.01b	0.28±0.02b	0.54±0.02a	0.24±0.01b
	2-pentyl furan	1013.50	612.40	2.02	0.02±0.01d	0.03±0.02b	0.03±0.01c	0.04±0.01d	0.06±0.01a	0.02±0.01b
	Total				0.31±0.02c	0.39±0.04b	0.40±0.02b	0.32±0.03c	0.60±0.03a	0.26±0.02d

The overall distribution of the different chemical classes according to the cultivar is reported in Figure 4-4. The 64 compounds identified were used to perform a hierarchical cluster analysis and the results were visualized using a heatmap (Figure 4-5). All the cultivars were well discriminated. The proximity of QYX and HTDZ was confirmed, although they were separated in the hierarchical cluster analysis. On the whole, esters (isoamyl acetate, ethyl acetate, ethyl propanoate, ethyl pentanoate, ethyl hexanoate, butyl acetate, ethyl 3-methylbutyrate, ethyl heptanoate, hexyl butanoate, propyl acetate, and ethyl 2-hydroxypropanoate) and ketones (acetoin, 6-methyl-5-hepten-2-one, 1-octen-3-one, 2-pentanone, acetone, 3-octanone, 2-hexanone, and 2-heptanone) were the more predominant VOCs of the six red jujube cultivars (Figure 4-4, Table 4-3).

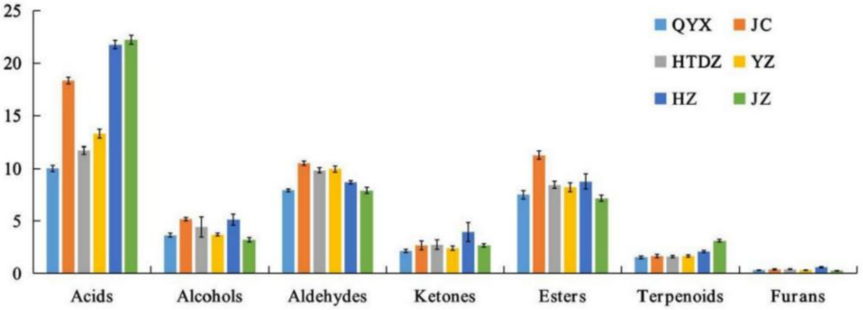


Figure 4-4 Semi-quantitative results of GC-IMS analysis of the volatiles in six red jujube cultivars achieved from Xinjiang province, China (Jinchang-‘JC’, Junzao-‘JZ’, Huizao-‘HZ’,Qiyuexian-‘QYX’, Hetiandazao-‘HTDZ’, and Yuanzao-‘YZ’)

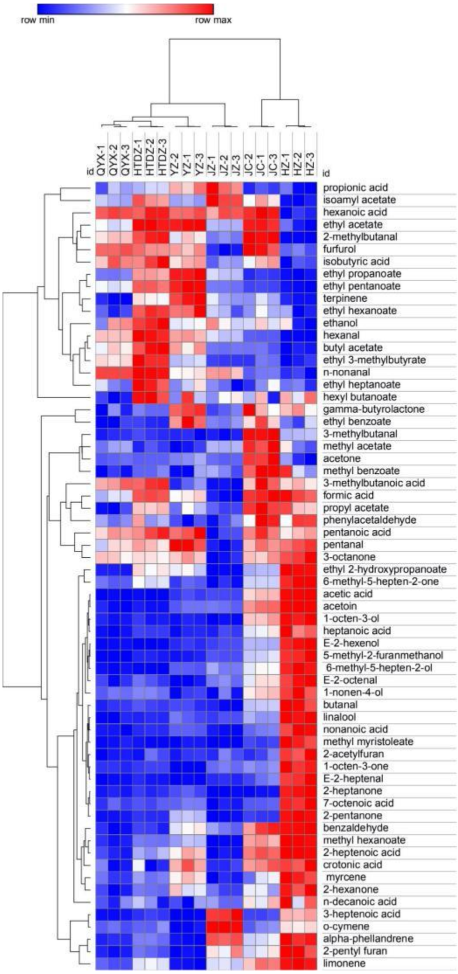


Figure 4-5 Heatmap analysis of volatiles got from GC-IMS of the six red jujube cultivars in Xinjiang province, China (Jinchang-'JC', Junzao-'JZ', Huizao-'HZ',Qiyuexian- 'QYX', Hetiandazao- 'HTDZ', and Yuanzao- 'YZ')

HZ had the highest content of ketones (17.01 μg/g) including acetoin, 1-octen-3-one, 6-methyl-5-hepten-2-one, acetone, 2-hexanone, linalool, 2-pentanone, and 3-octanone, followed by JC (13.76 μg/g). Acetoin acts as an aglycone and exists in glycosidically bound form in some fruits and vegetables (banana, lychee, durian and kiwi fruit flowers, etc.) with a pleasant yogurt creamy odor (Xiao & Lu, 2014). 1-octen-3-one was a result of the degradation of fatty acids like linoleic and linolenic acids, which also existed in red jujube (Cho et al., 2006; Song et al., 2019). It was noticed that 6-methyl-5-hepten-2-one with fruity and apple-like odor also existed in the odor profile of tea (Guo et al., 2021). Besides, the concentration of linalool (0.16 μg/g), nonanoic acid (1.24 μg/g), methyl myristoleate (0.58 μg/g), 2-acetylfuran

(0.54 μg/g), 1-octen-3-one (0.08 μg/g), (E)-2-heptenal (0.44 μg/g), 2-heptenone (1.00 μg/g), 7-octenoic acid (0.16 μg/g), and 2-petanone (0.84 μg/g) in HZ were the highest. Linalool (3, 7-dimethyl-1, 6-octadien-3-ol) was widely found in plants, presenting a floral scent (Hoshino et al., 2020). Furans are characterized by the caramel-like, sweet, fruity, and nutty odor descriptions. In particular, 2-acetylfuran has a sweet, almond, and cream odor (He et al., 2020). The (E)-2-alkenals including (E)-2-heptenal have been reported to contribute to the oxidative off-flavor (Ties & Barringer, 2012). 2-Heptanone was described as one of the characteristic aroma compounds of ripe fruit with cinnamon and sweet odor (Yang et al., 2019). JC was more abundant in the content of acetone (1.16 μg/g), methyl acetate (2.20 μg/g), 3-methyl butanal (0.77 μg/g), methyl benzoate (0.12 μg/g), and propyl acetate (2.42 μg/g). Esters (8.75 μg/g and 12.21 μg/g) were dominant in both JZ and YZ.

QYX and HTDZ share several key features in the composition of VOCs. Esters (9.05 and 12.06 μg/g) accounted for the highest proportion in aroma composition, followed by acids (7.16 and 8.23 μg/g), ketones (6.80 and 6.95 μg/g), aldehydes (5.21 and 6.13 μg/g), alcohols (2.74 and 2.98 μg/g), alkenes (1.84 and 2.49 μg/g), and furans (0.31 and 0.40 μg/g) in QYX and HTDZ, respectively (Figure 4, Table 3). The concentration of thirteen kinds of common VOCs in QYX and HTDZ was also comparable, including pentanoic acid (2.16 and 2.83 μg/g), ethanol (2.31 and 3.00 μg/g), isobutyric acid (0.58 and 0.55 μg/g), butyl acetate (0.54 and 0.61μg/g), hexanoic acid (both 0.26 μg/g), 3-methyl butanoic acid (0.14and 0.13 μg/g), hexanal (0.19 and 0.25 μg/g), ethyl 3-methylbutyrate (both 0.03 μg/g), n-nonanal (0.41 and 0.44 μg/g), furfurol (0.19 and 0.22 μg/g), 2-methyl butanal (0.53 and 0.61 μg/g), 3-octanone (both 0.03 μg/g), and pentanal (0.02 and 0.03 μg/g). Among these common VOCs, hexanoic acid and pentanoic acid were also found in grape and perceived as a sweat odor, n-nonanal as citrus and green odor, and furfurol as sweet odor (Zarifikhosroshahi et al., 2020). 3-methylbutanoic acid was identified as one of the most important odorants in chocolate with fruity and sweaty aroma (Seyfried &Granvogl, 2019). Isobutyric acid is always detected in milk products with rancid, butter, and cheese like odor (Jia et al., 2020). Ethanol was presented in food products with odor descriptions like alcohol, floral, ripe apple, and sweet (Gao et al., 2014). The presence of esters like ethyl 3-methylbutyrate and butyl acetate always contributed to the fruity notes (Renault et al., 2015).

The VIP scores were used to highlight the most discriminatory features that contribute to the clustering of the different cultivars. Acetoin, E-2-hexanol, hexanal, acetic acid, and ethyl acetate were all the components with VIP score > 1, indicating their crucial role in the discrimination of the different cultivars (Figure 4-6).

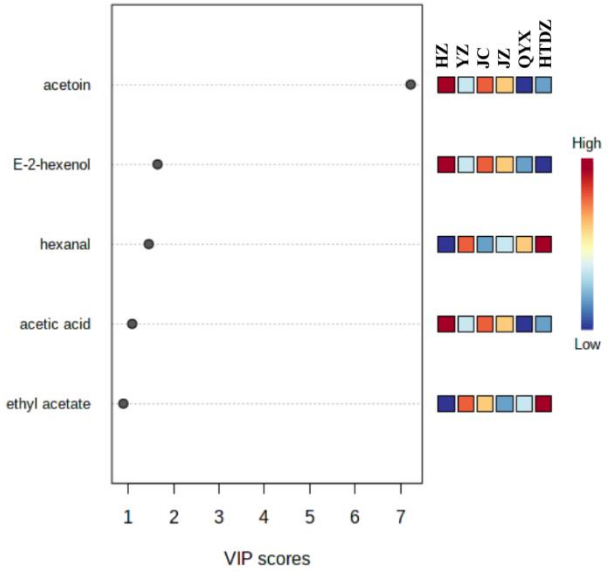


Figure 4-6 Volatiles with variable importance in projection (VIP) scores over 1 in the six red jujube cultivars from Xinjiang province, China (Jinchang- ‘JC’, Junzao- ‘JZ’, Huizao- ‘HZ’,Qiyuexian- ‘QYX’, Hetiandazao- ‘HTDZ’, and Yuanzao- ‘YZ’)

3.3. Fatty acids, amino acids, organic acids, and sugars

The composition of fatty acids, amino acids, organic acids, and sugars is reported in Table 4-4. The content of organic acids, including malic acid, citric acid, quinic acid, lactic acid, and tartaric acid in the six cultivars of red jujube was in the range of 22.87~159.01 mg/g. The amount of the three kinds of sugars (glucose, fructose, and maltose) was 20.59~126.02 mg/g. The concentration of fatty acids and amino acids were was in trace amounts (0~0.51 mg/g), except for proline (1.55~2.25 mg/g).

Table 4-4 The composition of fatty acids, amino acids, organic acids, and sugars of six red jujube cultivars from Xinjiang Province, China.

Composition	Concentration (mg/g)					
	Qiyuexian (QYX)	Jinchang (JC)	Hetiandazao (HTDZ)	Yuanzao (YZ)	Huizao (HZ)	Junzao (JZ)
malic acid	105.38±22.54a	106.01±18.34a	131.57±33.67a	145.47±43.23a	154.50±21.34a	159.01±54.42a
citric acid	22.87±3.46c	31.29±4.56bc	25.75±6.76bc	34.67±4.32b	23.96±3.50c	44.97±7.67a
quinic acid	91.37±10.22a	92.26±10.23a	100.67±12.89a	106.79±20.98a	111.19±23.56a	112.84±18.76a
lactic acid	56.38±6.44c	61.68±6.46c	85.59±7.74b	101.80±9.09ab	116.45±89.76a	121.84±20.03a
tartaric acid	55.49±9.87d	59.77±5.78d	86.97±6.43c	105.54±8.90b	118.82±9.76ab	123.57±12.65a
glucose	126.02±13.45a	84.70±7.65c	108.37±9.80b	87.22±6.73c	96.93±7.83bc	55.25±4.56d
fructose	150.67±1.24a	104.24±1.43d	121.68±1.23b	98.87±1.20e	118.61±41.10c	70.99±1.20f
maltose	28.64±3.00a	26.07±8.00a	27.71±5.00a	25.04±2.13a	26.15±2.23a	20.59±1.80a
capric acid	0.22±0.01c	nd	nd	0.35±0.01a	0.26±0.01b	0.26±0.01b
lauric acid	1.98±0.01b	0.81±0.01e	0.77±0.01f	2.91±0.01a	1.68±0.01c	1.21±0.01d
myristic acid	2.16±0.01b	0.74±0.01e	0.68±0.01f	3.44±0.02a	1.27±0.01c	1.16±0.01d

myristoleic acid	1.45±0.01b	0.54±0.01e	0.57±0.03d	5.08±0.01a	0.40±0.01f	1.02±0.01c
palmitic acid	12.30±0.01b	4.44±0.01f	4.67±0.01e	1.74±0.01a	5.47±0.01d	7.13±0.01c
palmitoleic acid	4.28±0.02b	2.22±0.02e	2.39±0.02d	6.35±0.02a	1.35±0.02f	3.91±0.02c
margaric acid	nd	nd	0.02±1.00E-4a	nd	nd	0.18±0.03b
stearic acid	2.32±0.03a	0.84±0.03d	0.73±0.03e	0.14±0.03b	0.76±0.03f	0.94±0.03c
oleic acid	5.89±0.02a	0.84±0.002e	0.79±0.02e	1.43±0.02d	0.37±0.02c	1.37±0.02b
linoleic acid	3.46±0.01a	0.71±0.01d	0.69±0.01d	0.77±0.01b	1.04±0.01d	1.52±0.01c
arachidic acid	0.34±0.01b	nd	nd	0.28±0.02c	nd	0.21±0.01a
alpha-linolenic acid	0.28±0.01b	0.17±0.01d	0.19±0.01d	0.20±0.01d	0.28±0.01c	0.47±0.01a
gamma-linolenic acid	nd	nd	nd	0.18±0.01a	nd	nd
behenic acid	0.27±0.01d	nd	nd	nd	0.26±0.01e	0.31±0.01c
lignoceric acid	nd	nd	nd	0.24±0.01a	nd	nd
ASP	0.044±0.0002c	0.036±0.0002b	0.033±0.0002a	0.053±0.0002e	0.021±0.0002f	0.042±0.0002d
THR	0.28±0.001d	0.51±0.001a	0.48±0.001b	0.17±0.001f	0.19±0.001e	0.34±0.001c
SER	nd	nd	nd	nd	nd	nd
GLU	0.011±0.0003c	0.032±0.0003e	0.026±0.0003e	0.0035±0.0003b	0.013±0.0003d	0.02±0.0003a
GLY	0.0049±0d	0.0055±0a	0.0059±0b	0.0031±0f	0.003±0e	0.0043±0c

ALA	0.015±0.0001c	0.028±0.0001d	0.026±0.0001b	0.0051±0.0001e	0.008±0.0001b	0.023±0.0001a
VAL	0.0053±0d	0.042±0a	0.037±0b	0.01±0e	0.014±0d	0.028±0c
MET	nd	nd	nd	nd	nd	nd
ILE	0.03±0.0001d	0.015±0.0001b	0.015±0.0001a	0.047±0.0001e	0.029±0.0001c	0.051±0.0001b
LEU	0.0024±0c	0.019±0e	0.015±0e	0.0016±0b	0.002±0d	0.0087±0a
TYR	0.018±0.0002d	0.013±0.0002a	0.021±0.0002b	0±0.0002f	0.021±0.0002e	0.04±0.0002c
PHE	0.0093±0.0001c	0.022±0.0001c	0.021±0.0001b	0±0.0001c	0.0092±0.0001b	0.014±0.0001a
LYS	0.0007±0d	0.0043±0a	0.0039±0b	nd	0.025±0d	0.039±0c
HIS	nd	0.01±0c	0.0093±0d	nd	nd	0.0031±0a
ARG	0.023±0.0001d	0.047±0.0001b	0.058±0.0001a	nd	0.028±0.0001c	0.047±0.0001b
PRO	1.70±0.12b	1.64±0.12b	1.55±0.12b	2.25±0.12a	1.66±0.12b	2.17±0.12a

‘nd’ means ‘not detected’. Mean values with different letters in the same row correspond to significant differences at $p < 0.05$. Data are represented as the mean ± SD.

3.4. Correlation analysis between sensory attributes and chemical composition

The sensory profiles of the six red jujube cultivars is displayed in Figure 4-7. All the six red jujube cultivars were characterized by higher values on jujube ID (7.0~8.0), sweet (6.4~7.8), and fruity attributes (5.8~6.4). The sour (1.9~3.2), floral (0.6~1.5), bitter (0.4~0.8) and astringent attribute (0.3~1.0) were relatively lower in the red jujubes, compared with the aforementioned three attributes. Besides, HZ was more prominent in fruity (6.4) and floral (1.5) attributes. JZ had a higher value on sweet (7.8), sour (3.2) and jujube ID (8.0) attributes. HTDZ got higher scores in bitter (0.8) and astringent (1.0) attributes. Furthermore, JC, QYX and YZ showed higher values in jujube ID (8.0), sweet (7.2) and sour (3.0), respectively.

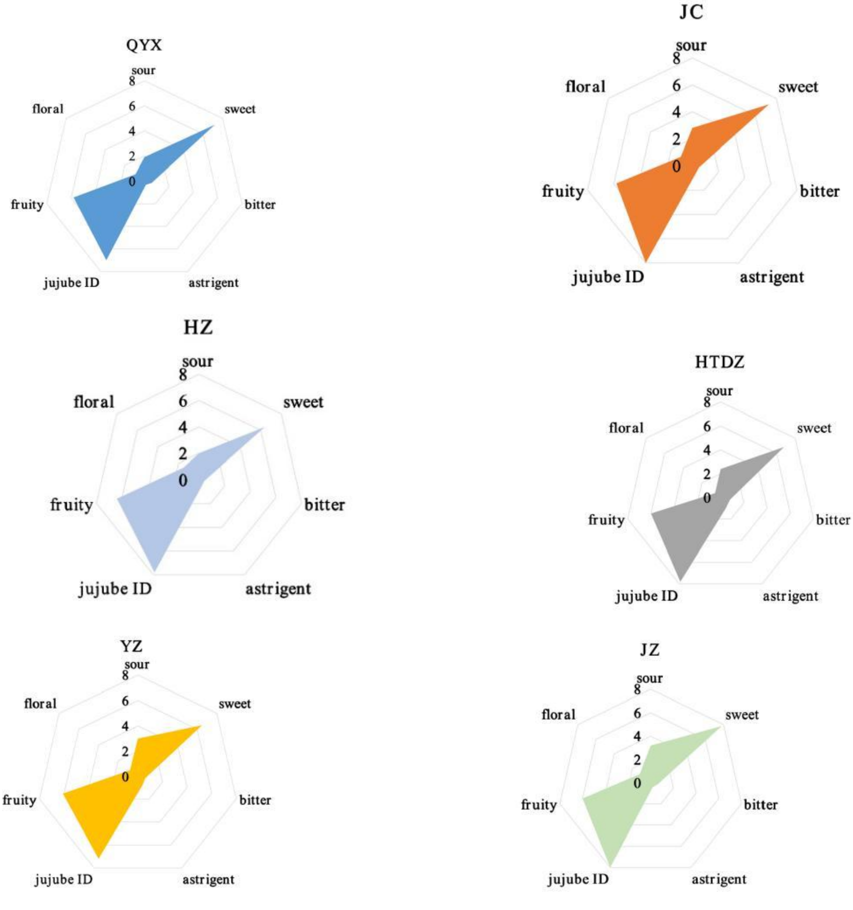


Figure 4-7 Sensory profiles of the six red jujube cultivars in Xinjiang province, China (Jinchang-‘JC’, Junzao-‘JZ’, Huizao-‘HZ’,Qiyuexian-‘QYX’, Hetiandazao-‘HTDZ’, and Yuanzao-‘YZ’)

The correlation analysis between sensory characteristics and all VOCs, fatty acids, amino acids, organic acids, and sugars had also been carried out. Redundancy analysis (RDA) was carried out to analyze the relationship between chemistry composition (factors) and sensory values. The findings seemed cannot be explained well in a logical way. The correlation between the sensory profile and the chemical composition (limiting to VOCs with VIP score > 1, as they played an important role in the differentiation of the cultivars, they were further applied for the data mining) is reported in Figure 4-8. Floral attribute was in high correlation with VOCs (acetoin, E-2-hexenol, and acetic acid). The fruity attribute was correlated with organic acids (malic acid, quinic acid, lactic acid, and tarctic acid). Jujube ID was mainly correlated with the presence of amino acids (THR, GLU, GLY, ALA, VAL, LEU, TYR, PHE, LYS, HIS, ARG). This finding could be correlated with the phenomenon that free amino acids may transaminate or dehydrogenate into aldehydes or acids (Schwab et al., 2008). For example, phenylacetaldehyde and isobutanoic acid could be issued from the oxidation of PHE and VAL, separately (Jelen, 2011). Thus, phenylacetaldehyde and isobutanoic acid may be the contributors to the jujube ID. The concentration of MET and SER in fresh jujube (Junzao cultivar) at red maturity were 0.58 mg/100g and 36.91 mg/100g, respectively (Song et al., 2019). However, MET and SER were not detected in the six cultivars of red jujubes, suggesting that they might gradually decompose completely and form sulfur-containing substances, amine compounds, aldehydes, and ketones during the natural drying process (from fresh jujubes to red jujubes) through deamination and decarboxylation reactions. The astringency attribute was correlated only with hexanal and ethyl acetate. The astringency was perceived as a comprehensive feeling of roughing, drying, shrinking or drawing, which could increase and last for a longer time after swallowing (Ishikawa&Noble, 1995). Phenolic components, multivalent salts like alum, organic acids, and charged polysaccharides such as chitosan can stimulate astringency (Peleg, 1998; Rodriguez et al., 2003). The astringent attribute is thus a complex physicochemical reaction among volatile, non-volatile chemicals, and chewing, thus explaining the low correlation with the compounds here examined. The bitter attribute was correlated with hexanal and margaric acid. Sweet attribute is covaried with amino acids (THR,GLU,GLY,ALA, VAL, LEU, TYR, PHE, LYS, HIS, ARG). Amino acids were perceived as one or more tastes according to their structures. In the cases of L-/D-ARG and L-/D-HIS, the main taste evaluated was as sweet and/or bitter. The cases of GLY, THR and ALA were expected to be sweet in red jujubes. VAL, LEU and PHE were evaluated as a sweet and bitter taste in D-form (Kawai et al., 2012). Sour attribute was in close relationship with organic acids (malic acid, citric acid, quinic acid, lactic acid and tartaric acid), amino acids (ILE and PRO) and some fatty acids. Among the five correlated organic acids, citric acid could contribute much to a sour gustatory flavor quality as it is to lemon (Penniston et al., 2008). However, L-ILE and D-PRO were evaluated as bitter taste (Kawai et al., 2012). As a result, the taste was a complex effect of the structural properties of amino acids, which could be explained by the fact that different structures of amino acids were perceived by multiple taste receptors (Malnic et al., 1999; Araneda et al., 2000).

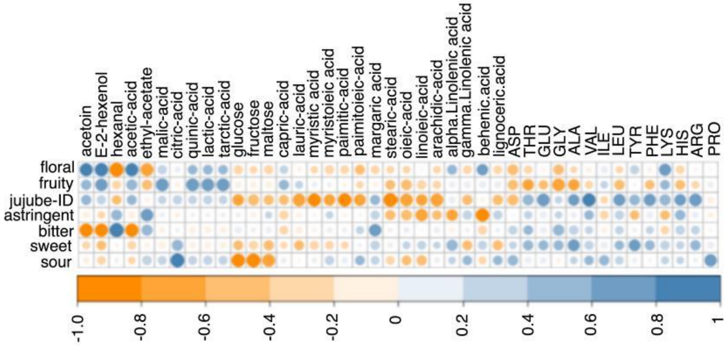


Figure 4-8 Correlation analysis among sensory attributes and fatty acids, amino acids, organic acids, sugars and VOCs (VIP score＞1) of the six red jujube cultivars from Xinjiang province, China (Jinchang-'JC', Junzao-'JZ', Huizao-'HZ',Qiyuexian -'QYX', Hetiandazao-'HTDZ', and Yuanzao-'YZ')

4. Conclusion

The volatile profiles of six red jujube cultivars originated from Xinjiang province, China, were analyzed by E-nose and GC-IMS. The chemical composition of the non-volatile metabolites, i.e. fatty acids, amino acids, organic acids, and sugars, along with a sensory evaluation were also determined. Descriptive sensory analysis and multivariate data analysis were carried out to correlate the sensory perception with the chemical fingerprinting. A total of sixty-four kinds of VOCs have been identified in the six red jujube cultivars (HZ, JZ, YZ, JC, HTDZ and QYX). Esters and acids were the more predominant VOCs. Acetoin, E-2-hexanol, hexanal, acetic acid, and ethyl acetate played an important role in the discrimination of the six red jujube cultivars. The differences in sensory attributes of the red jujubes could be explained by the composition of VOCs, amino acids, fatty acids, and organic acids to some extent.

5

ChapterⅤ. Elucidation of Baking Induced Changes in Key Odorants of Red Jujube Using Gas Chromatography-Olfactometry, Flavor Dilution Factors, Odor Activity Values, Recombination, and Omission Analysis

As a homology of medicine and food, red jujube was cooked in ways of baking, steaming, boiling, and frying in daily life. The aroma of red jujube produced during baking was unique, attractive, and easy to recognize which was attributed to thermal reactions like caramelization and non-enzymatic Maillard reaction. However, the particular aroma of baked red jujube has not been evaluated and elucidated until now. The objective of this study was to reveal the key odor active compounds' changes in the baking process of red jujube.

Qiao, Y., Chen, Q., Gou, M., Zhang, J., Purcaro, G., Jin, X., Wu, X., Lyu, J., and Bi, J. (2022). Elucidation of Baking Induced Changes in Key Odorants of Red Jujube Using Gas Chromatography-Olfactometry, Flavor Dilution Factors, Odor Activity Values, Recombination, and Omission Analysis. Intended journal: *Journal of Agricultural and Food Chemistry*. Under review.

Abstract

Jujube is a fruit widely consumed fresh or used as ingredient in food industry, especially in China. Processed red jujubes is characterized by a caramel-like and roasted aroma which contrasted with the sweet and fruity aroma of raw red jujube. The characteristic volatiles that contribute to this aroma have never been elucidated. The volatile organic components (VOCs) of baked and raw red jujubes were determined based on the molecular sensory science including gas chromatography-mass spectrometry-olfactometry (GC-MS-O), flavor dilution (FD) factors, odor activity value (OAV), recombination, and omission experiments. Results revealed that *β*-damascenone, 5-butyltetrahydro-2-furanone, and hexanoic acid were the common key odor active compounds in raw and baked red jujube. However, the baking process triggered the formation of aromatic hydrocarbon molecules in red jujube including 1H-pyrrole-2-carboxaldehyde (0.21 μg/g) and 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (34.15 μg/g), especially 5-methyl-2-furancarboxaldehyde (0.36 μg/g) and benzaldehyde (0.11 μg/g) that contributed the most to the caramel-like and roasted aroma of baked red jujube, respectively.

Keywords: Red jujube, Baking, GC-MS-O, Recombination and omission test, 5-Methyl-2-furancarboxaldehyde

1. Introduction

Red jujube (*Ziziphus Jujuba* Mill.), originated from China, is a member of the rhamnaceae botanical family (Zhang et al., 2021). Red jujube can be consumed fresh or added as an ingredient of other products after processing. The processing methods of red jujube mainly include steaming, boiling, and baking (Liu et al., 2016). After baking, red jujube can be use in products like jujube tea and cakes, which are popular among Chinese consumers. The aroma of baked red jujube is unique, attractive, and easy to recognize, and it is attributed to thermal reactions like caramelization and non -enzymatic Maillard reaction (Kays & Wang, 2000). For example, furanic compounds such as furfural generated in the Maillard reaction might impact burnt/cooked and roasted aromas in bakery products and smoked products. (Srivastava et al., 2018; Varlet et al., 2006). According to the descriptive analysis of aroma, the characteristics of red jujube (fresh) in aspects of sour, sweet, jujube ID (a sweet and fruity flavor associated with jujube), fruity, and floral were evaluated (Galindo et al., 2015). However, the particular aroma of baked red jujube has never been evaluated and elucidated until now.

The aroma perception is a comprehensive result of certain chemical stimuli on the sensations of smell, taste, and trigeminus nerve (Schieberle &Hofmann, 2011). It is recognized that a single molecule does not generate the aroma perception characteristic of foods but it is given by the combination of multiple components in specific concentration (Schieberle &Hofmann, 2011). Molecular sensory science, or sensomics, is the discipline introduce by Schieberle and co-workers to define the key-aroma components. Molecular sensory science combines the information acquired by GC-MS-O, flavor dilution (FD) factors, odor activity value (OAV), aroma recombination, and aroma omission tests, is the most effective way for the clarification of the key aroma compounds of foods. (Schieberle & Hofmann, 2011;Jelen, 2012; Song & Liu, 2018). In order to elucidate the effect of chemical stimulus on olfaction, gas chromatography-mass spectrometry-olfactometry (GC-MS -O) is used to fingerprint, qualify, and describe the characteristics of VOCs in foods (Giungato et al., 2018).There are three ways to determine the FD factor: I) dilution by varying the sample volume; II) dilution by diluting the sample; III) dilution through adjusting GC injector split ratio (Feng et al., 2015). OAV is the ratio of the concentration of a certain compound to the odor threshold. The combination of OAV and FD factor can reflect the contribution of the target chemical stimulus to the overall aroma. The recombined model is a mixture of compounds with OAV ≥ 1 and/or FD factor ≥ 1, which was conducted to compare its aroma characteristics with those of the target sample. Omission experiments are carried out to prove the importance of a specific compound or class of compounds for a particular aroma characteristics, by removing one or a class of aroma compounds one by one and evaluating the aroma by a trained panel (Jelen, 2012; Zhu & Xiao, 2018). Molecular sensory science has been successfully applied in the identification of key odor active compounds that are responsible for the aroma of different fruits (cranberry, mulberry, jujubes) and processed foods (peas, rapeseeds) (Zhu et al., 2016; Zhu et al., 2018; Bi et al., 2020; Ortner et al., 2016; Zhu & Xiao, 2018).

In relation to red jujube, hexanal, (E)-2-octenal, *β*-damascenone, ethyl hexanoate, 3-mercaptohexyl acetate, and 2,5-dimethylpyrazine were identified as the key odor-active compounds in different cultivars of fresh fruits, i.e. Jinsixiaozao, Youzao, and Yuzao (Zhu et al., 2018). However, the key odorants of red jujube induced by baking has never been reported.

The objective of this study was to reveal the key odor active compounds' changes in the baking process of red jujube (*Ziziphus Jujuba* Mill. cv. Junzao) by odor description analysis, FD factors, quantitative analysis, OAV, recombination tests, omission tests, and quantitative descriptive analysis (QDA).

2. Material and methods

2.1. Material and reagents

Red jujube (*Ziziphus. jujuba* Mill. cv.'Junzao') was purchased from Populus Fruit Industry Co., Ltd., Alaer city, Xinjiang province, China (2019). The jujubes were stored at 4 °C until being tested.

C4 - C20 alkanes mixture was purchased from O2si Smart Solution (Charleston, West Virginia, USA). The following standards were all of GC grade. Octanoic acid (99%) and 2-cyclohexenone (95%) were purchased from MilliporeSigma (St. Louis, MO, USA). Hexanoic acid (99%) was purchased from Ehrenstorfer GmbH, Augsburg, Germany. N-hexane (99%), pentanoic acid (99%), heptanoic acid (98%), trimethyl-pyrazine (99%), 4-hydroxybutanoic acid lactone (98%), heptanal (95%), limonene (95%), benzaldehyde (98%), acetic acid (99%), benzeneacetaldehyde (97%), *β*-damascenone (98%), acetoin (97%), and ethyl benzoate (99%) were purchased from TCI Development Co., Ltd., Shanghai, China. 5-Methyl-2-furancarboxaldehyde (98%), 5-ethyltetrahydro-2-furanone (98%), 1-acetyl-2-methylcyclopentene (96%), 5-butyltetrahydro-2-furanone (98%), linalool (97%), and 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP) (95%) were purchased from A ChemTek, Inc., Massachusetts, USA. 1H-pyrrole-2-carboxaldehyde (98%) was purchased from Panphy Chemicals Corporation, LOS Angeles, USA. (E)-linalool oxide (furanoid) (98%) was purchased from ANPEL Laboratory Technologies Inc., Shanghai, China. Alcohol of analytical grade was purchased from Tong Guang Fine Chemicals Company, Beijing, China. Pure water was achieved by a Milli-Q purification system (Millipore, Waltham, MA, USA).

2.2. Sample preparation

Preparation of raw red jujube sample. About 500 g of red jujube (cv. Junzao) without pests and diseases were cleaned, kernel removed, sliced (4 mm), frozen by liquid nitrogen and ground for 90 s with Joyoung grinder (JYL-CO20, Joyoung Co., Ltd., Shandong, China).

2.3. Baking condition

Two variables (baking time and temperature) and their interactions with the color acceptance and the caramel aroma intensity of red jujube (cv. Junzao) were evaluated by three sensory panelists. The two variables ranged from 150 to 200 ℃ for baking temperature and 3 to 5 min for baking time. Both color acceptability and burnt aroma intensity were rated from 0 to 10, where 0 was unacceptable, 5 was acceptable, and 10 was liked.

2.4. Preparation of baked red jujube sample

Red jujube slices were put into an ovenware tray and baked at 180 °C for 4 min in an isothermal forced-air oven after the preheating of the oven for 20 min at 180 °C (PT 2531, Media Co., Ltd., Anhui, China). The baked slices were frozen by liquid nitrogen immediately and ground for 90 s into powder with Joyoung grinder.

Accurate 2.0 g of the grounded raw and baked red jujube was put into a 20 mL vial sealed with a magnetic screw-cap and septum, separately. A volume of 4 mL sodium chloride solution (4 mol/L) was added to the vials to make it a uniform system before testing.

2.5. Sensory evaluation

Aroma profiles of raw and baked red jujube samples were evaluated by QDA. The sensory evaluation of red jujube was referred to the study conducted by Wojdyło et al., 2016. Twelve panelists (7 females and 5 males, aged between 24 and 35 years-old) recruited from fruit and vegetable processing team with rich experience in fruit sensory evaluation discussed the sensory attributes of raw and baked red jujube and reached a consensus. There were six sensory attributes agreed to describe the sensory characteristics, including sweet, sour, fruity, floral, caramel, and roast. The solutions prepared from the following compounds were in concentrations of 50-fold above their individual odor threshold (500 mg/L benzyl alcohol (sweet=7), 3.75 mg/L acetic acid (sour=4), 13.25 mg/L ethyl benzoate (fruity=5), 0.5 mg/L linalool (floral=5), 25 mg/L 4-hydroxybutanoic acid lactone (caramel=4), and 100 mg/L trimethyl-pyrazine (roast=7) dissolved in water with addition of 5% alcohol, separately) (Zhai & Granvogl, 2020).

The aroma intensity was quantified with a numerical scale where 0 represented none and 10 represented extremely strong with an increment of 0.5. For the evaluation, fifteen jujube slices for each panelist were served in the testing room of 26 °C. Each sample was evaluated in triplicate with a 10 min wait.

2.6. Headspace solid-phase microextraction (HS-SPME) GC-MS-O analysis

HS-SPME Conditions. A RTC120 HS-SPME autosampler from Guangzhou Zhida Laboratory Technology Co., Ltd., was applied for the extraction of VOCs from the samples. The vials were firstly incubated at 60 °C for 15 min with a shaking speed of 450 rpm. A smart SPME arrow fiber of divinylbenzene/carbonWR/polydimethylsiloxane (DVB/CAR/PDMS) (120/20 μm film thickness, 1.5 mm inner diameter, CTC Analytics AG) was applied for the adsorption of VOCs from the samples. The fiber was pre-conditioned at 250°C for 30 min before use. The extraction condition was set as 60 °C for 40 min.

2.7. GC-MS-O analysis

A GC-MS (8890-5977B, Agilent Technologies, Inc., Santa Clara, CA, USA) in combination with an olfactory detector port (OP275, GL Sciences, Inc., Tokyo, Japan) and DB-WAX column (60 m × 0.32 mm, 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA, USA) were used. Helium (99.999%) was used as the carrier gas with a flow rate of 1mL/min. The temperature of the injector, the MS interface, and the transferline were all at 230 ° C. The extracted VOCs were desorbed at the injection port of GC for 2 min. The oven program started at 40 °C for 2 min, arrived at 230 °C with a ramp of 5 °C/min , and held for 7 min. The GC column effluent was split to the MS and olfactory port in a 1:4 ratio by a 4-port splitter (SilFlow, Trajan, Australia). The splitter was flushed with helium at 5 mL/min. The electron-impact mass spectra was generated at 70 eV with a scan range from 35 to 350 *m/z*, ion source temperature was 230 ° C and the temperature of quadrupole was 150 °C. Humidified air was provided to the sniffing port at a rate of 15 mL/min to keep a relatively comfortable humidity for the nasal mucosa. Three experienced panelists consisting of one female and two males (age 20–35) took part in the olfactory tests. The total sniffing time was 47 min. The odor descriptions were determined when at least two panelists confirmed the results. Similar odor descriptions of the VOCs were discussed, summarized and agreed upon all panelists, in comparison with the information from the LRI & Odour Database - Odour Data, VCF home, and Flavornet. All samples were tested in triplicate.

2.8. Qualitative and quantitative analysis

For qualitative analysis, mass spectra of the VOCs were firstly compared with that in NIST 17 library. The RI got from NIST 17 library was then compared with that calculated from n-alkanes (C4 - C20) with deviations less than 10. Moreover, the VOCs would be confirmed with the retention time of reference standards.

For quantitative analysis, the addition of internal standard (2-cyclohexenone, 1 μL, 100 ppm) was used for semi-quantitative analysis of VOCs at first. Secondly, the quantitation of VOCs with FD factors ≥ 1 was carried out using the standard addition method (Brown & Mustoe, 2014). Standard addition is one of the extrapolation techniques showing superiority when the concentration of the analyte is large and when the analytical technique employed is sensitive to the matrix (Brown & Mustoe, 2014). It has been successfully applied in the determination of caffeine in drinks (Tobolkina et al., 2014). In this study, five points were selected separately for the standard curve of the targeted VOCs using the standard addition method and the quantitative analysis was performed according to the quantifier ions defined in each mass spectrum. The calibration equations and quantifier ions are available in Table 5-2.

2.9. FD factors and OAV

The split ratio was increased from 1:2, 1:8, 1:26, to 1:80, corresponding to FD factors of 3, 9, 27 and 81, respectively. The FD factor of a certain VOC was determined until it was not detected in GC-MS-O. The OAVs were calculated according to the quantitation results together with the odor thresholds of the VOCs.

2.10. Recombination and omission tests

To prepare the non-flavor matrix, raw and baked red jujube powder were extracted with pentane, methanol, n-hexane, and ethanol stepwise for 36 h with a ratio of 1:6 (m/V), respectively. The residues were dried for 3 days at 25 °C after filtration. Then the obtained matrix was pre-frozen with liquid nitrogen, and lyophilized (Bafangzhongda LGJ-10B) at 50 °C for 36 h (cold trap temperature was -40 °C) until nothing was detected by sensory evaluation, HS-SPME-GC-MS-O, and HS-SPME-GC-MS(Ren et al., 2021). A mixture of all quantitated odorants with FD and OAV $\geq$ 1 in their naturally occurring concentrations was added to the odorless matrix of raw and baked red jujube to set up the recombinates, respectively. Then the recombinates were compared with the original raw and baked red jujube according to the principle of triangle test, separately(UNI ISO 4120 Norm).

Omission tests were also carried out, referred to the triangle test. The recombinates were set up by omitting the VOCs (FD and OAV $\geq$ 1) one by one. Then the sensory notes of the recombinates with certain VOCs omitted were compared with the recombinates with all odorants (FD and OAV $\geq$ 1) to clarify the correlation of the certain VOCs on the corresponding sensory notes.

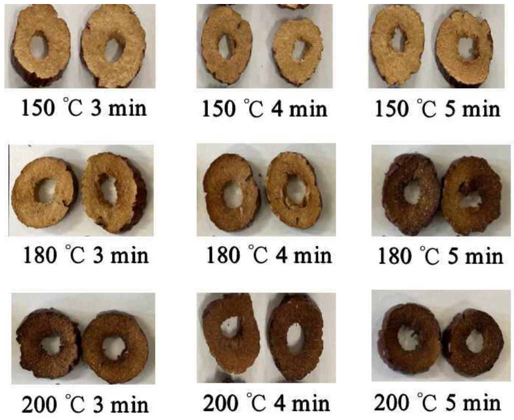
2.11. Statistical analysis

Data standardization and significance analysis was performed using SPSS 13.0 software (SPSS Inc, Chicago, IL).

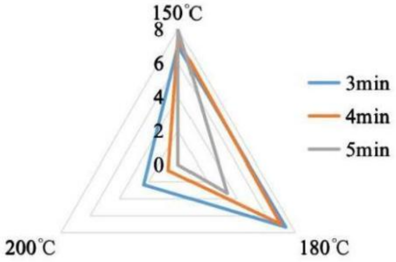
3. Results and discussion

3.1. Baking condition

The effect of baking temperature and baking time was investigated and optimized via sensory evaluation. As were shown in Figure 5-1, the highest and lowest values of the acceptance of the color were obtained separately at the baking condition of 150 °C, 5min (the acceptance of the color was 8.0), and 200 °C, 5min (the acceptance of the color was 0). The highest value of the intensity of the caramel aroma was achieved at the baking condition of 180 °C, 4min (the intensity of the caramel aroma was 8.3), 200 °C, 4min (the intensity of the caramel aroma was 8.3), and 200 °C, 5min (the intensity of the caramel aroma was 8.3). The higher baking temperature would enhance the caramel aroma intensity and lead to the unacceptable color. Combining these two indicators, the higher color acceptance and caramel aroma intensity could be achieved at the baking condition of 180 °C, 4min (the acceptance of the color was 7.0 and the intensity of the caramel aroma was 8.3).


150 ℃ 3 min
150 ℃ 4 min
150 ℃ 5 min
180 ℃ 3 min
180 ℃ 4 min
180 ℃ 5 min
200 ℃ 3 min
200 ℃ 4 min
200 ℃ 5 min

(a)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3min
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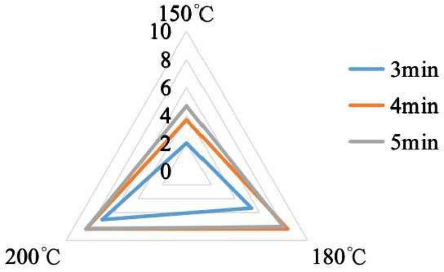
(b)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180℃
200℃
3min
4min
5min

(c)

Figure 5-1 (a) The pictures of different baking conditions (150 °C~180 °C; 3-5min); (b) The radar plot of the color acceptance of red jujube slices under different baking conditions (150 °C~180 °C; 3-5min); (c) The radar plot of the caramel aroma intensity of red jujube slices under different baking conditions (150 °C~180 °C;3-5min).

3.2. Sensory evaluation

Sensory evaluation of raw and baked red jujubes is presented in Figure 5-2. Sour (3.0), sweet (7.0), fruity (8.0), and floral (2.0) were the main attributes of red jujube (*Ziziphus jujuba* Mill. cv.'Junzao'). The highest intensities were observed for sweet and fruity attributes, followed by sour and floral ones, which were consistent with the previous study.Compared with red jujubes, sweet (9.0) was still the main attribute in baked red jujube, however, caramel (8.0), and roast (7.0) notes presented a higher score as well. The formation of roasted and caramel-like aroma after the baking process was similar to the results also observed in beans and hazelnut (Rainey, 1986; Bi et al., 2021).

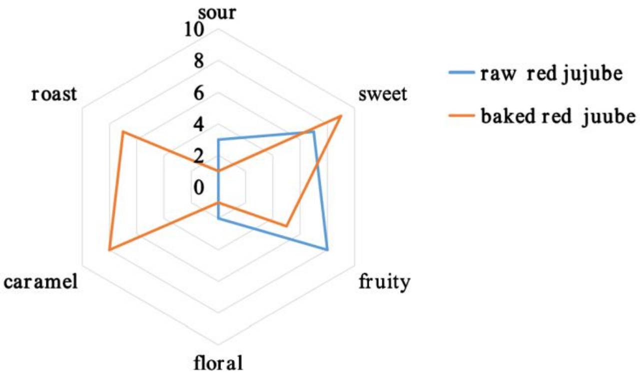


Figure 5-2 Sensory evaluation results of raw and baked red jujube

3.3. VOCs identified by GC-MS-O

The VOCs of raw and baked red jujube were extracted by HS-SPME and identified by GC-MS-O. As shown in Table 5-1, seventy VOCs were identified in raw red jujube, composed by aldehydes (14), ketones (13), pyrazines (8), acids (8), esters (8), alcohols (4), alkenes (4), pyrroles (3), furans (6), heterocyclic compounds (1), and benzenes (1). For baked red jujube, there were sixty-seven kinds of VOCs including aldehydes (13), ketones (13), pyrazines (8), acids (8), esters (7), alcohols (3), alkenes (2), pyrroles (3), furans (7), heterocyclic compounds (1), thiophene (1), and pyran (1). Acid was the major chemical class of raw (76.47%) and baked (71.54%) red jujube. Meanwhile, aldehyde ranked the second major chemical class with a percentage of 8.34% and 7.51% in raw and baked red jujube correspondingly.

In terms of the semi-quantitative results (Table 5-1), the content of aldehydes, ketones, pyrazines, acids, esters, alcohols, alkenes, and bicyclic compounds decreased in the baked red jujube. Nevertheless, the concentration of pyrroles, thiophenes, and pyrans increased after the baking process. In detail, the concentration of 2-acetylpyrrole (0.022 μg/g), 1H-pyrrole-2-carboxaldehyde (0.075 μg/g), 2-hexylthiophene (0.011 μg/g), and DDMP (0.320 μg/g) were higher in baked red jujube than those in raw red jujube. The increase in the content of these heterocyclic compounds was probably due to the Maillard reaction of sugars and amino acids in red jujube during thermal processing(Alasalvar et al., 2003). In addition, DDMP might also be formed by polymerization of the cleavage products from glucose (Li et al., 2019).

Table 5-1 Volatile organic compounds in raw and baked red jujube identified using HS-SPME-GC-MS-O.

Classes	Compound	Linear retention index		Identification^a	Concentration (μg/g)^b		Flavor dilution factor	
		RI (literature)	RI (calculated)		Raw red jujube	Baked red jujube	Raw red jujube	Baked red jujube
aldehydes(14)	3-methylbutanal	915	916	RI,MS	0.052±0.003	0.038±0.002	nd.	nd.
	hexanal	1080	1077	RI,MS	0.037±0.001	0.020±0.002	nd.	nd.
	heptanal	1184	1179	RI,MS,S,O	nd.	0.037±0.002	1	1
	trans-2-hexenal	1212	1215	RI,MS	0.046±0.0008	0.038±0.003	nd.	nd.
	octanal	1287	1281	RI,MS	0.034±0.006	0.029±0.003	nd.	nd.
	trans-2-heptenal	1320	1319	RI,MS	0.038±0.002	0.029±0.002	nd.	nd.
	nonanal	1390	1385	RI,MS	0.092±0.005	0.086±0.003	nd.	nd.
	trans-2-octenal	1424	1423	RI,MS	0.091±0.001	0.083±0.006	nd.	nd.
	decanal	1483	1488	RI,MS	0.018±0.003	0.015±0.0008	nd.	nd.
	benzaldehyde	1520	1519	RI,MS,S	1.251±0.003	0.57±0.007	nd.	1
	trans-2-nonenal	1532	1528	RI,MS	0.018±0.003	nd.	nd.	nd.
	5-ethylcyclopent-1-	none	1635	MS	0.040±0.001	0.020±0.0003	nd.	nd.

	enecarboxaldehyde							
	benzeneacetaldehyde	1646	1640	RI,MS,S,O	0.101±0.023	0.112±0.014	1	nd.
	2-phenylcrotonaldehyde	1947	1944	RI,MS	0.012±0.0007	0.007±0.0009	nd.	nd.
	total				1.830±0.077	0.972±0.046		
ketones (13)	1-(3-methylenecyclopentyl)-ethanone	none	1120	MS	0.042±0.0008	0.022±0.001	nd.	nd.
	1-(1,4-dimethyl-3-cyclohexen-1-yl)-ethanone	1145	1141	RI,MS,O	0.177±0.002	0.102±0.009	3	nd.
	acetoin	1287	1284	RI,MS,S,O	0.145±0.007	0.063±0.004	3	3
	hexanone	1291	1292	RI,MS	0.048±0.005	0.024±0.0009	nd.	nd.
	6-methyl-5-hepten-2-one	1339	1330	RI,MS	0.068±0.041	0.016±0.0003	nd.	nd.
	6-methyl-3,5-heptadiene-2-one	1582	1587	RI,MS	0.066±0.001	0.027±0.003	nd.	nd.
	1-acetyl-2-methylcyclopentene	none	1607	MS	0.070±0.006	0.031±0.003	3	3
	acetophenone	1655	1651	RI,MS	0.024±0.001	0.019±0.002	nd.	nd.
	4-hydroxyheptanoic acid lactone	1817	1813	RI,MS	0.094±0.003	0.054±0.002	nd.	nd.
	β-damascenone	1835	1831	RI,MS,S,O	0.052±0.005	0.042±0.002	81	81
	nerylacetone	1855	1858	RI,MS	0.021±0.001	0.016±0.0008	nd.	nd.
	4-nonanolide	2055	2049	RI, MS	0.146±0.006	0.074±0.006	nd.	nd.

	2-pentadecanone	2131	2133	RI,MS	0.013±0.0004	0.010±0.0006	nd.	nd.
	total				0.966±0.079	0.500±0.035		
pyrazines (9)	methylpyrazine	1266	1267	RI,MS	0.006±0.002	nd.	nd.	nd.
	2,6-dimethylpyrazine	1328	1328	RI,MS	0.052±0.003	0.027±0.003	nd.	nd.
	2,3-dimethylpyrazine	1346	1346	RI,MS	nd.	0.006±0.0006	nd.	nd.
	2-ethyl-6-methylpyrazine	1385	1383	RI,MS	0.182±0.023	0.045±0.004	nd.	nd.
	trimethylpyrazine	1400	1402	RI,MS	0.052±0.0008	0.024±0.002	nd.	nd.
	2,6-diethylpyrazine	1432	1431	RI,MS	0.053±0.004	0.021±0.0009	nd.	nd.
	2,3-dimethyl-5-ethylpyrazine	1460	1459	RI,MS	0.060±0.001	0.028±0.0016	nd.	nd.
	tetramethylpyrazine	1466	1473	RI,MS	0.029±0.0008	0.015±0.0003	nd.	nd.
	2-methyl-5-(1-propenyl) pyrazine	none	1656	MS	0.030±0.0002	0.012±0.001	nd.	nd.
	total				0.464±0.035	0.178±0.013		
acids (8)	isovaleric acid	1684	1686	RI,MS	0.184±0.004	0.138±0.007	nd.	nd.
	acetic acid	1449	1440	RI,MS	1.937±0.113	1.081±0.03	nd.	nd.
	pentanoic acid	1750	1759	RI,MS,S	0.441±0.022	0.248±0.004	nd.	1
	hexanoic acid	1849	1851	RI,MS,O	4.58±0.144	2.673±0.194	3	3
	heptanoic acid	1968	1967	RI,MS,S,O	4.325±0.052	2.464±0.17	3	3

	octanoic acid	2084	2081	RI,MS,S,O	4.521±0.190	2.309±0.143	1	1
	nonanoic acid	2211	2212	RI, MS	0.443±0.053	0.229±0.024	nd.	nd.
	2-octenoic acid	none	2314	MS,S,O	0.342±0.024	0.112±0.007	1	1
	total				16.773±0.602	9.254±0.579		
esters (8)	methyl caprylate	1378	1380	RI,MS	0.048±0.002	0.015±0.0003	nd.	nd.
	trans-methyl-2-octenoate	1484	1491	RI,MS	0.020±0.0004	nd.	nd.	nd.
	methyl caprate	1586	1584	RI,MS	0.103±0.002	0.056±0.004	nd.	nd.
	methyl benzoate	1614	1620	RI,MS	0.134±0.004	0.044±0.002	nd.	nd.
	methyl dodecanoate	1805	1799	RI,MS	0.127±0.007	0.088±0.001	nd.	nd.
	methyl hydrocinnamate	1853	1854	RI,MS	0.040±0.003	0.019±0.0007	nd.	nd.
	methyl tetradecanoate	2005	2014	RI,MS,S	0.013±0.002	0.010±0.001	nd.	nd.
	methyl myristoleate	2052	2056	RI,MS	0.054±0.004	0.026±0.004	nd.	nd.
	total				0.539±0.024	0.258±0.013		
alcohols (4)	2-methyl-1-butanol	1207	1206	RI,MS	0.013±0.0004	nd.	nd.	nd.
	terpinen-4-ol	1601	1598	RI,MS	0.027±0.0002	0.014±0.0006	nd.	nd.
	alpha-terpineol	1692	1694	RI,MS	0.036±0.003	0.019±0.001	nd.	nd.
	benzyl alcohol	1886	1883	RI,MS	0.078±0.003	0.052±0.006	nd.	nd.

	total				0.154±0.007	0.085±0.0076		
alkenes (4)	beta-myrcene	1156	1151	RI,MS	0.009±0.0008	0.005±0.0004	nd.	nd.
	limonene	1192	1189	RI,MS,S,O	0.212±0.015	0.083±0.002	1	3
	crithmene	1240	1236	RI,MS	0.013±0.002	nd.	nd.	nd.
	styrene	1245	1251	RI,MS	0.122±0.010	nd.	nd.	nd.
	total				0.356±0.028	0.088±0.0024		
pyrroles (3)	1-ethyl-2-formylpyrrole	1610	1608	RI,MS	0.020±0.001	0.017±0.0004	nd.	nd.
	2-acetylpyrrole	1975	1983	RI,MS	0.014±0.0004	0.022±0.001	nd.	nd.
	1H-pyrrole-2-carboxaldehyde	2048	2041	RI,MS,S,O	0.010±0.0008	0.075±0.034	nd.	9
	total				0.220±0.007	0.826±0.091		
furans (7)	2-pentylfuran	1229	1222	RI,MS	0.009±0.0003	0.008±0.0006	nd.	nd.
	(E)-linalool oxide (furanoid)	1464	1466	RI,MS,S,O	0.016±0.005	0.009±0.0005	1	1
	3-furaldehyde	1455	1455	RI,MS	0.196±0.005	0.165±0.013	nd.	nd.
	5-methyl-2-furancarboxaldehyde	1560	1567	RI,MS,S,O	0.094±0.002	0.053±0.004	3	27
	acetylfuran	1498	1498	RI,MS	nd.	0.007±0.0008	nd.	nd.
	5-ethyltetrahydro-2-furanone	1708	1706	RI,MS,S,O	0.043±0.001	0.025±0.0003	3	3
	5-butyldihydro-2(3H)-furanone	1934	1932	RI,MS,S,O	0.100±0.004	0.059±0.0006	1	nd.

	total				0.458±0.018	0.326±0.030		
bicyclic compounds (1)	cyclohexanone ethylene acetal	none	1398	MS	0.142±0.009	0.118±0.010	nd.	nd.
benzenes (1)	p-xylene	1130	1131	RI,MS	0.033±0.002	nd.	nd.	nd.
thiophenes (1)	2-hexylthiophene	none	1956	MS	nd.	0.011±0.001	nd.	nd.
pyrans (1)	3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one)(DDMP)	2295	2291	RI,MS,S,O	nd.	0.320±0.020	nd.	27

‘a’ retention index was calculated from n-alkanes with the deviation within 10.
‘b’ concentration was expressed as relative quantitative results using 2-cyclohexenone as internal standard.
‘nd.’ means not detected.

3.4. FD factor analysis

Further analysis of VOCs was conducted considering both odor description and FD factors, the number of odor active compounds with FD factors ≥ 1 in raw and baked red jujube were 15 and 16, separately (Table 5-1).

The predominant common odor-active compounds in raw and baked red jujubes were ketones (3), acids (4), furans (3), aldehydes (1), and alkenes (1) including acetoin (FD=3 and 3), 1-acetyl-2-methylcyclopentene (FD=3 and 3), *β*-damascenone (FD=81 and 81), hexanoic acid (FD=3 and 3), heptanoic acid (FD=3 and 3), octanoic acid (FD=1 and 1), 2-octenoic acid (FD=1and 1), (E)-linalool oxide (furanoid) (FD=1 and 1), 5-methyl-2-furancarboxaldehyde (FD=3 and 27), 5-ethyltetrahydro-2-furanone (FD=3 and 3), heptanal (FD=1 and 1), and limonene (FD=1 and 3) determined by GC-MS-O) (Table 5-1).

Ketones are formed in the metabolism of carbohydrates like the anaerobic decomposition of cellulose, starch, hemicellulose, and pectin, usually reported as sweet odors(Rosenfeld et al., 2021). Among which, *β*-damascenone has the highest FD factor (81) in both raw and baked red jujube, which means that it had a very important influence on the aroma of both. It has also been identified as one of the key odor-active compounds in dried red jujubes by other authors (*Ziziphus jujuba* Mill. cv.'Jinsixiaozao', 'Youzao', and 'Yuzao') (Zhu et al., 2018b). The five compounds including acetoin (butter, cream), 1-acetyl-2-methylcyclopentene, hexanoic acid (acid), heptanoic acid (sour), and 5-ethyltetrahydro-2-furanone (herb like) had a lower FD factor (3) in both raw and baked red jujube, ranking the second class in the effect of the aroma. Another group of four compounds including octanoic acid, 2-octenoic acid, (E)-linalool oxide (furanoid), and heptanal had the lowest FD factor (1) in both raw and baked red jujube, meaning their smallest influence on the whole aroma. Hexanoic acid and octanoic acid were two of the aroma-impact compounds of jujube that were consistent with the previous studies (Wang et al., 2018; Song et al., 2019). However, the VOCs of 5-methyl-2-furancarboxaldehyde and limonene, which were identified in raw red jujube before (*Ziziphus Jujuba* Mill. cv. 'Junzao'), had higher FD factors in baked red jujube (27 and 3) than those in raw red jujube (3 and 1) (Pu et al., 2018).

Through the baking process, the distinctive aroma-active compounds in raw red jujube including benzeneacetaldehyde, 5-butyldihydro-2(3H)-furanone, and 1-(1,4-dimethyl-3-cyclohexen-1-yl)-ethanone were disappeared, while benzaldehyde, 1H-pyrrole-2-carboxaldehyde, and DDMP were identified as the newly formed odor active compounds in baked red jujube. Benzeneacetaldehyde is an aromatic aldehyde that is also reported in black tea. Combined with the results of the olfactometry, it was found that benzeneacetaldehyde contributed 'fruity and berry' notes to red jujube, while benzaldehyde contributed 'burnt sugar and cherry' notes to baked red jujube. 1H-pyrrole-2-carboxaldehyde was identified as an informative roasting indicator of roasted hazelnut with a burnt odor (Pu et al., 2018). DDMP, as a bitter taste compound, was also widely found in thermal treated common products, like coffee and bread (Li et al., 2021b).

3.5. Quantitation results and calculation of OAV

The quantitation of VOCs with FD factors ≥1 was carried out by adding the standards into the raw and baked red jujube matrix. The standard curves, correlation coefficients, quantitation results, as well as OAVs are shown in Table 5-2.

Table 5-2 Quantitative results of volatile organic compounds with flavor dilution factors ≥1

Compounds	CAS	Quantifier ions	Raw red jujube		Baked red jujube		Odor threshold in water (μg/g)[b]	Odor description [a,b,,g]	Raw red jujube	OAV	Baked red jujube	OAV
			Calibration equations	R^2	Calibration equations	R^2			Concentration (μg/g)		Concentration (μg/g)	
heptanal	111-71-7	114	y=5.380x+2.922	0.9921	y=6050.8x+79218.1	0.9909	0.003	citrus, green [a]	0.54	181.06	0	0
limonene	138-86-3	136	y=4.024x+65615.4	0.9917	y=6.127x+552969	0.9933	0.070	lemon, orange [a,g]	0.02	0.23	0.09	1.29
acetoin [f]	513-86-0	/	/	/	/	/	0.850	butter, cream [a,b,g]	0.15 [f]	0.17	0.06[f]	0.07
(E)-linalool oxide (furanoid) [f]	34995-77-2	/	/	/	/	/	0.190	citrus, flower, fresh, lemon [a,g]	0.02 [f]	0.08	0.01 [f]	0.04
benzaldehyde	100-52-7	106	/	/	y=9.573x+1.085	0.9903	0.10	burnt sugar, cherry [a,b]	0	0	0.11	1.14
1-acetyl-2-methylcyclopentene	3168-90-9	109	y=2.528x+48182.5	0.9913	y=3.404x+44332.4	0.9988	not found	sweet [g]	0.002	none	0.001	none

5-methyl-2-furancarboxaldehyde	620-02-0	110	$y=7.017x+1.161$	0.9790	$y=4.674x+1.698$	0.9951	0.500	sweet, caramel, cooked, burnt sugar [a,g]	0	0	0.36	0.73
benzeneacetaldehyde	122-78-1	120	$y=243613x+524387.7$	0.9943	/	/	0.004	fruity, berry, nut, pungent [a,g]	2.15	538.14	0	0
5-ethyltetrahydro-2-furanone	695-06-7	85	$y=2.513x+750848.2$	0.9979	$y=1.665x+643346.5$	0.9976	0.260	herb, sweet, fruity [a,b,g]	0.30	1.15	0.39	1.49
pentanoic acid	109-52-4	60	$y=2.513x+6.665$	0.9952	$y=2.154x+2.052$	0.9900	1.370	sour, cheese, pungent, putrid, sweat [a,g]	2.65	1.94	0.95	0.70
β-damascenone	23726-93-4	190	$y=891.1x+26805.2$	0.9930	$y=770.7x+26784.4$	0.9949	0.0001[h]	sweet, apple, rose, honey[a,g]	30.08	300810	34.75	347500
hexanoic acid	142-62-1	87	$y=1.357x+8.264$	0.9753	$y=820953x+4.965$	0.9941	3.000	acid, cheese, rancid,sweat[a]	6.09	2.03	6.05	2.02
5-butyldihydro-	104-	85	$y=1.670x+981044$	0.998	$y=1.086x+640232$	0.998	0.014	fruit，	0.06	4.20	0.06	4.21

2(3H)-furanone	50-7		.1	6		1		coconut [a,g]				
heptanoic acid	111-14-8	101	y=2.251x+2.149	0.9902	y=769640.4x+1.619	0.9921	1.330	rancid, sour, sweat[a]	1.82	1.37	2.10	1.58
octanoic acid	124-07-2	115	y=1.016x+1.448	0.99301	y=319270x+1.101	0.9832	19.00	acid, rancid, sweat[a,g]	1.43	0.08	0.78	0.04
1H-pyrrole-2-carboxaldehyde	1003-29-8	95	/	/	y=410334.6x+84740.4	0.9955	65.0	burnt [g]	0	0	0.21	0.003
DDMP [d]	28564-83-2	54.6	/	/	y=0.390x+0.027 [e]	0.9920	2.060	bitter [c]	0	0	34.15 [e]	16.58

Note:

‘a’ represents Flavornet.

‘b’ represents VCF Home.

‘c’ represents the reference ‘Li, H.; Zhang, W.; Tang, X.; Wu, C.; Zhao, Z. Identification of bitter-taste 425 compounds in class-iii caramel colors. Flavour Fragr. J. 2021,36, 404–411.’

‘d’ represents DDMP(2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one).

‘e’ quantified by HS SPME GC-MS/MS in previous study but not published.

‘f’ the results was the semi-quantified results according to Table 1.

‘g’ odor quality detected at the sniffing port.

‘h’ represents the reference ‘Zhu, J.; Xiao Z. Characterization of the major odor-active compounds in dry jujube cultivars by application of gas chromatography-olfactometry and odor activity value. J. Agric. Food Chem. 2018, 66, 7722–7734.’

‘/’ means no information.

No corresponding standards or odor thresholds for 1-(1,4-dimethyl-3-cyclohexen-1-yl)-ethanone, 1-acetyl-2-methylcyclopentene, and 2-octenoic acid were available, thus they could not be quantified by standard addition method and no OAVs could be evaluated.

Among the seventeen quantitated compounds, *β*-damascenone showed the highest concentration in raw red jujube with 30.08 μg/g, followed by hexanoic acid (6.09 μg/g). The concentration of benzeneacetaldehyde, heptanoic acid, and octanoic acid were at the level of 1~3 μg/g. Heptanal, 5-methyl-2-furancarboxaldehyde, and 5-ethyltetrahydro-2-furanone were present in lower amounts (0~1 μg/g). Meanwhile, limonene, 1-acetyl-2-methylcyclopentene, and 5-butyldihydro-2(3H)-furanone were detected in traces (≤58 ng/g). For baked red jujube, the concentration of *β*-damascenone was 34.75 μg/g, which was a little bit higher than that in raw red jujube. Moreover, the content of DDMP, newly formed in the baking process, was 34.15 μg/g. The amounts of hexanoic acid and heptanoic acid were lower (1~3 μg/g). Limonene, benzaldehyde, 1-acetyl-2-methylcyclopentene, 5-methyl-2-furancarboxaldehyde, 5-ethyltetrahydro-2-furanone, pentanoic acid, 5-butyltetrahydro-2-furanone, octanoic acid, and 1H-pyrrole-2-carboxaldehyde were only determined in the range of 1ng/g to 950 ng/g.

In terms of the calculation of OAVs, the number of compounds with OAVs ≥ 1 was eight in both raw and baked red jujube (Figure 5-3). *β*-Damascenone had the highest value of 300810, followed by benzeneacetaldehyde (538.14), heptanal (181.06), 5-butyldihydro-2(3H)-furanone (4.20), hexanoic acid (2.03), pentanoic acid (1.94), heptanoic acid (1.37), and 5-ethyltetrahydro-2-furanone (1.15) in raw red jujube. These compounds were associated with odors described as sweet, fruity, citrus, green, coconut, sour, and herb-like notes.

The highest OAVs in baked red jujube were obtained as well for *β*-damascenone (347500), followed by DDMP (16.57), 5-butyldihydro-2(3H)-furanone (4.21), hexanoic acid (2.01), heptanoic acid (1.582), 5-ethyltetrahydro-2-furanone (1.49), benzaldehyde (1.14, and limonene (1.29). These key aroma active compounds presented sweet, bitter, fruit, sour, herb, burnt sugar, lemon, and orange-related notes.

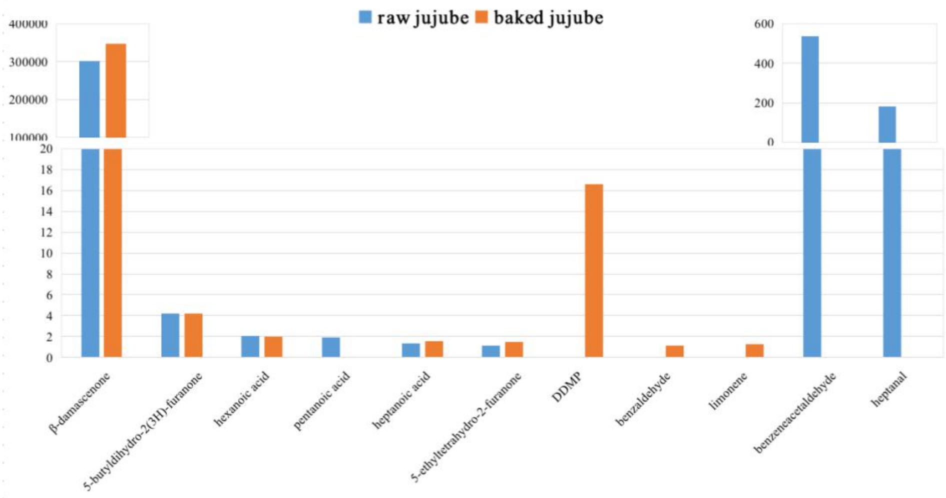
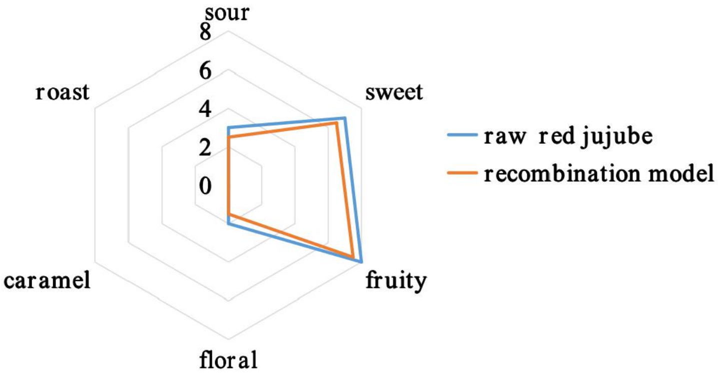


Figure 5- 3 Sensory evaluation results of the recombinates of raw red jujube (a) and baked red jujube (b) compared with their original samples.

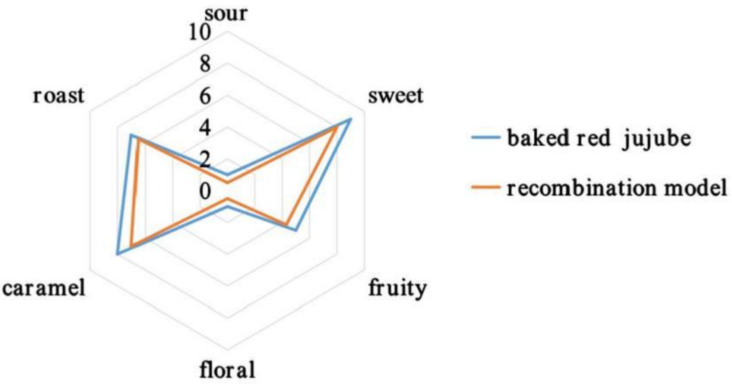
In addition, some VOCs with FD factor≥1 identified in raw and baked red jujube showed OAVs <1, like limonene, acetoin, (E)-linalool oxide (furanoid), 5 -methyl-2-furancarboxaldehyde, 5-ethyltetrahydro-2-furanone in raw red jujube, and heptanal, acetoin, (E)-linalool oxide (furanoid), 5-methyl-2-furancarboxaldehyde, 1H-pyrrole-2-carboxaldehyde, and octanoic acid in baked red jujube. Besides, 5-butyldihydro-2(3H)-furanone was not identified in the olfactometry test of baked red jujube, but presented an OAV of 4.21 after quantitation. Therefore, the contribution of the above mentioned VOCs to the aroma of the samples was disputable. This phenomenon may due to: i) the threshold was determined in water, but when the characteristics and intensity of volatilization from the real matrix were screened out, it may cause sensory interactions with a variety of elements such as sugar, protein, vitamins, etc.; ii) the baking induced changes in the texture of red jujube might cause bias in the volatility of VOCs, which in turn affects the evaluation from panelists (Marcinkowska et al., 2021). Thus, it is important to observe the FD factors and OAVs in the substantiation of odor-active compounds.

3.6. Recombination tests

The recombination tests were carried out by mixing the VOCs with OAV ≥ 1 according to their concentrations in Table 5-2 into the non-flavor matrix of raw and baked red jujube, separately. The compounds with sub-threshold concentrations may also have an impact on the whole aroma (Guadagni et al., 1963). Thus, 5-methyl-2-furancarboxaldehyde was also added to the non-flavor matrix of baked red jujube to comprehensively consider its contribution to the aroma perception, combining its FD factor (FD=27 in baked red jujube) and odor description of sweet, caramel, cooked, and burnt sugar detected at the sniffing port, though its OAV is lower than 1. The sensory evaluation results are shown in Figure 5-4. It can be clearly seen that the intensities of the six sensory attributes of the recombinates of raw and baked red jujube were similar to those of the original raw and baked red jujube, confirming that the key odor active compounds of raw red jujube were *β*-damascenone, benzeneacetaldehyde, heptanal, 5-butyltetrahydro-2-furanone, hexanoic acid, pentanoic acid, heptanoic acid, and 5-ethyltetrahydro-2-furanone, and those of baked red jujube were *β*-damascenone, DDMP, 5-butyltetrahydro-2-furanone, hexanoic acid, heptanoic acid, 5-ethyltetrahydro-2-furanone, benzaldehyde, limonene and 5-methyl-2-furancarboxaldehyde. Therefore, the results showed that the typical aromas of raw red jujube and baked red jujube had been successfully simulated, which was further in accordance with the identification and quantitative results.



(a)



(b)

Figure 5-4 Sensory evaluation results of the recombinates of raw red jujube (a) and baked red jujube (b) compared with their original samples.

3.7. Omission test

Omission tests were carried out by omitting certain compounds of the recombinaiton models one by one, to verify the importance of the contribution to overall aroma according to the recombination tests. The contribution of each compound was evaluated by the twelve panelists through triangle tests.

As was shown in Table 5-3, heptanal, benzeneacetaldehyde, and β-damascenone showed a very high significance ($p \leq 0.001$), indicating their great contribution to the overall aroma of raw red jujube. The model lacking hexanoic acid and 5-butyltetrahydro-2-furanone separately showed a high significant difference ($p \leq 0.05$), compared with the recombination model. The omission of pentanoic acid also resulted in a significant difference. However, 5-ethyltetrahydro-2-furanone and heptanoic acid contribute little to the overall aroma of raw red jujube. At last, the key odor active compounds of red jujube were confirmed as heptanal, benzeneacetaldehyde, β-damascenone, hexanoic acid, 5-butyltetrahydro-2-furanone, and pentanoic acid.

Table 5-3 The results of omission tests for raw and baked red jujube.

Samples	Model	Odorants omitted from the recombination model	Number [a]	Significance [b]
Raw red jujube	1	heptanal	10	***
	2	benzeneacetaldehyde	12	***
	3	5-ethyltetrahydro-2-furanone	3	

	4	pentanoic acid	8	*
	5	*β*-damascenone	11	***
	6	hexanoic acid	9	**
	7	5-butyldihydro-2(3H)-furanone	9	**
	8	heptanoic acid	2	
Baked red jujube	1	limonene	8	*
	2	benzaldehyde	9	**
	3	5-methyl-2-furancarboxaldehyde	10	***
	4	5-ethyltetrahydro-2-furanone	2	
	5	*β*-damascenone	12	***
	6	hexanoic acid	8	*
	7	5-butyltetrahydro-2-furanone	8	*
	8	heptanoic acid	2	
	9	DDMP	8	*

‘a’ : Number of correct judgments from twelve panelists.

‘b’: ‘*’ significant ($p \leq 0.05$); ‘**’ highly significant ($p \leq 0.01$); ‘***’very highly significant ($p \leq 0.001$).

‘DDMP’ was short for ‘2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one’.

In terms of omission tests for baked red jujube, the models without 5-methyl-2-furancarboxaldehyde and *β*-damascenone separately resulted in a very highly significant difference in the aroma perception of baked red jujube ($p \leq 0.001$). The omission of benzaldehyde showed a high significant difference ($p \leq 0.01$). The contribution of limonene, hexanoic acid, 5-butyltetrahydro-2-furanone, and DDMP were also significant compared with the recombinated model ($p \leq 0.05$), whereas the models lacking 5-ethyltetrahydro-2-furanone, and heptanoic acid were not differentiated from the recombination model. Thus the key odor active compounds of baked red jujube were 5-methyl-2-furancarboxaldehyde, *β*-damascenone, benzaldehyde, limonene, hexanoic acid, 5-butyltetrahydro-2-furanone, and DDMP.

3.8. Changes of key odor active compounds after baking

As mentioned above, the key odor active compounds that both existed in raw and baked red jujube were *β*-damascenone, 5-butyltetrahydro-2-furanone, and hexanoic acid. Hexanoic acid and 5-butyltetrahydro-2-furanone were firstly identified as odor active compounds associated with jujube or processed jujube. The concentration of 5-butyltetrahydro-2-furanone and hexanoic acid in both raw and baked red jujube was close to each other, leading to the OAV of 4.2 and 2.0, respectively (Table 5-2). *β*-Damascenone presented the highest OAV among the key odor active compounds of both raw and baked red jujube, which indicated its significant influence on the overall aroma. *β*-Damascenone was reported to be generated from the degradation and cleavage of carotenoids, as well as thermal degradation of neoxanthin (Yang et al., 2013; Sefton et al., 2011).The increase of *β*-damascenone in the baking process of red jujube was also found in the thermal treatment of apple, coffee, and tea, which also indicated that the formation of *β*-damascenone could be enhanced by heat treatment (Schreier et al., 1978; Czerny & Grosch, 2000; Kumazawa & Masuda, 2001)

Benzeneacetaldehyde, heptanal, and pentanoic acid were confirmed as the specific odor active compounds of raw red jujube. Meanwhile, the odor description of benzeneacetaldehyde and pentanoic acid was fruity and sour, which might lead to the stronger intensity of fruity and sour attributes in sensory quality. Benzeneacetaldehyde is a product of Maillard reaction, which might be formed by the Amadori rearrangement, enolization, and Strecker degradation of the compounds with carbonyl and amino groups in raw red jujube (Liu et al., 2021). However, benzeneacetaldehyde was no longer detected in the baked samples (Table 5-2), which might because of its conversion to the corresponding acid during thermal processing (Wibowo et al., 2015). A similar thermal-induced decrease in the concentration of benzeneacetaldehyde was also observed in sugarcane juice heated at 90~110 ° C(Wang et al., 2020). Heptanal was also identified as one of the odor active compounds with citrus and green odor in jujube puree (Zhu et al., 2018b). Pentanoic acid existed in raw red jujube (Junzao) with sour odor description.

Limonene, benzaldehyde, 5-methyl-2-furancarboxaldehyde, and DDMP were identified as the specific odor active compounds of baked red jujube, of which 5-methyl-2-furancarboxaldehyde might contribute more to the caramel attribute, benzaldehyde contributed to the roast related aroma. As one of the terpenes that widely existed in nature, limonene was quantified both in raw and baked red jujube (Vieira et al., 2018).However, the concentration of limonene in baked red jujube was over five times that in raw red jujube. Thus limonene contributed more to the aroma of the baked samples. Benzaldehyde was identified as one of the odor active compounds in red jujube (*Ziziphus Jujuba* Mill. *cv.*'Jinsixiaozao', 'Youzao', and 'Yuzao') (Zhu et al., 2018b). However, it was not detected in the cultivar of 'Junzao'(Table 5-2). Benzaldehyde was quantified as 0.11 μg/g in the baked samples and contributed to the burnt sugar flavor. As one of the products deriving from roasting process, 5-methyl-2-furancarboxaldehyde has also been identified in the roasted palm kernels with odor description of caramel, burnt sugar, and roasted related aroma, expected to bring about changes in sensory attributes of caramel and roast (Zhang et al., 2017; Zhang et al., 2018).The formation of 5-methyl-2-furancarboxaldehyde was attributed to the reaction process of dehydration, halogenation, and hydrogenation of carbohydrates like fructose, which might also be the reason for the formation of 5-methyl-2-furancarboxaldehyde in baked red jujube (Feng et al., 2015; Li et al., 2007). DDMP, an o-heterocyclic compound, formed from the cleavage and polymerization of glucose or the 2,3-enolization from 1-deoxyglucosone in the Maillard reaction, is widely distributed in thermal treated food like heated pear, roasted palm kernel, and prune, etc. (Zhang et al., 2018; Hwang et al., 2013; Čechovská et al., 2011). In terms of odor description, DDMP was taste bitter when it was accompanied by impurities formed from glucose (Li et al., 2019). However, DDMP derived from maltol could also be tasteless as recently reported by Chen et al. (Chen et al., 2021).

4. Conclusions

Seventy and sixty-seven VOCs were identified in raw and baked red jujube, respectively, by HS-SPME-GC-MS-O. The molecular sensory science of sensory evaluation, FD factors, OAVs, recombinaiton test, and omission test were applied for the identification of key odor active compounds. Six compounds including heptanal, benzeneacetaldehyde, *β*-damascenone, hexanoic acid, 5-butyltetrahydro-2-furanone, and pentanoic acid contributed significantly to the overall aroma of raw red jujube, while 5-methyl-2-furancarboxaldehyde, *β*-damascenone, benzaldehyde, limonene, hexanoic acid, 5-butyltetrahydro-2-furanone, and DDMP were the key odorants for baked red jujube. The baking process enhanced the intensity of sweet notes, and gave rise to the notes of caramel and roast related, which might be caused by the existence of 5-methyl-2-furancarboxaldehyde and benzaldehyde.

6

Chapter Ⅵ. Rapid and Sensitive Quantitation of DDMP (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one) in Baked Red Jujubes by HS-SPME-GC-MS/MS

DDMP is thought to have bitter taste and a negative consequence on animal health. The quick, easy, cheap, effective, rugged, and safe sample preparation and HPLC-MS method for the detection of DDMP in beverages was based on solvent assistant extraction, reached a LOD of 1.00 µg/g, just slightly lower than the odor threshold of DDMP in water (2.06 µ g/g). Therefore, the aim of this part is to develop a sensitive, accurate, easy to operate, and solvent - free method using HS-SPME-GC-MS/MS for the detection of DDMP and apply the method for the quantification of DDMP in thermal processed red jujube. Furthermore, the correlated factors for the formation of DDMP will be analyzed to better understand the formation pathway of DDMP and control its' formation.

Qiao, Y., Bi, J., Chen, Q., Wu, X., Jin, X., Gou, M., Yang, X., & Purcaro, G. Rapid and sensitive quantitation of DDMP (2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one) in baked red jujubes by HS-SPME-GC-MS/MS. *Food Control*, 2022, 135, 108820.

Abstract

Baked red jujube is a common food ingredient used to enrich the nutritional value and enhance the aroma. DDMP (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one) with intense bitterness and potential toxicity may be present in several baked red jujube-based products but there are no well-defined methods to measure it quickly, sensitively, and accurately. Headspace solid-phase microextraction with gas chromatography-tandem mass spectrometry (HS-SPME-GC-MS/MS) was applied for the analysis of DDMP. The standard curve was linear between the concentrations of 0.01 and 100 μg/mL, with a limit of detection at 0.1 ng/g that below the sensory threshold of DDMP (2.06 μg/g). Five cultivars of baked red jujubes from China were evaluated with the method for DDMP content (4.55 ~ 46.50 μg/g). The results indicated that jujube cultivars with a higher molar ratio of proline to glucose, lower moisture content, and stronger ability to resist water stress should be avoided from baking to modulate the formation of DDMP.

Keywords:DDMP, Baking, Red jujube, HS-SPME-GC-MS/MS, Correlation analysis

1. Introduction

Red jujube (*Ziziphus jujuba* Mill.) represents a healthy food choice due to the high content of biologically active components such as phenolic compounds, triterpenic acids, polysaccharides, etc., which are correlated to both high nutritional values and biological activities, including antioxidant, anti-inflammatory, and antimicrobial activities (Gao, Wu & Wang, 2013; Kalogeropoulos et al., 2010; Feng et al., 2019; Gbaguidi et al., 2015). Red jujube is extensively cultivated in tropical and subtropical regions, such as East Asia (China), South Asia (India), North Africa, and Middle Eastern countries, and is now gradually being cultivated worldwide, including Russia, southern Asia, southern Europe, southwestern United States, and Australia (Rashwan et al., 2020). China is the native region of red jujube and over 400 cultivars are available (Li et al., 2007). Red jujube is also used as food ingredient (e.g., in breads, cakes) thus it can undergo thermal processing (involving the Maillard reaction), contributing to the formation of a particularly strong aroma (Rashwan et al., 2020).

Among the formed compounds, DDMP (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one), formed after 2,3-enolization and Amadori rearrangement products oxidation during baking of red jujube, is characterized by an intense bitter odor and potential toxicity (Li et al., 2019a). DDMP could also be found in various foods such as heated pear, whole wheat bread, and prunes (Hwang et al., 2013; Bin & Peterson, 2016; Cechovska et al., 2011; Lucie et al., 2011). The formation of DDMP was found to be related to sugars like glucose and fructose, amino acids including proline, tyrosine, histidine, and lysine (Zhou et al., 2014). Among these, the molar ratio of proline to glucose was important to the formation of DDMP (Nishibori & Kawakishi, 1994; Zhou et al., 2014). Furthermore, the factors of moisture content and reaction intensity of glucose and proline in the glucose-amino acids Maillard reaction models were also involved in the contribution to the formation of DDMP as reported by Li et al.(2019a).

Although the toxicological relevance of DDMP is not clear, it is thought to have a bitter taste and a negative consequence on animal health. DDMP was firstly identified as a novel nonenzymatic browning product using IR spectroscopy and mass spectrometry (Mills & Hodge, 1970). It was identified in the model reaction of amino acids and sugars, using gas chromatography mass spectrometry (GC-MS) (Kim, & Baltes, 1996). Recently, a method based on extraction in hexane for 30 min and 20% aqueous ethanol for 8h followed by GC-MS was applied for the determination of DDMP in wheat bread (Cechovska et al., 2011). The sample preparation was time-consuming, reaching an LOD of 1.00 μg/g, just slightly lower than the odor threshold of DDMP in water (2.06 μg/g) (Li et al., 2019b; Li et al., 2019c). Therefore, the development of a more sensitive, accurate, easy to operate, and solvent - free method for the detection of DDMP in food samples would be highly desirable.

GC coupled with tandem mass spectrometry (MS/MS) can provide the sensitivity and selectivity required with minimal sample preparation (Yost & Fetterolf, 1983). GC-MS/MS has been successfully applied in the fields of aroma and pesticide in food (Kind et al., 2017; Hopfer et al., 2016; Armorini et al., 2016; Zheng et al., 2020). For example, the detection of 2-acetyl-1-pyrroline in rice kernels, thymol and carvacrol in milk and tissues, 3-monochloro-1, 2-propanediol ester, 2-monochloro-1, 3-propanediol ester, glycidyl ester in fatty food, and pesticides in pigeonpea grains (Hopfer et al., 2016; Armorini et al., 2016; Zheng et al., 2020; Naik R. et al., 2021). However, there was no investigation carried out on the qualitative and quantitative characterization of DDMP in thermal processed red jujube.

This study aims to develop a sensitive, accurate, easy to operate, and solvent - free method using HS-SPME-GC-MS/MS for the detection of DDMP in thermal processed red jujube. The entire method was validated with regard to specificity, linearity, sensitivity, accuracy and precision using the matrix-matched method. This method was successfully applied to determine the quantity of DDMP in five cultivars of baked red jujubes.

2. Material and methods

2.1. Chemicals

Certified chemical standards of DDMP (A Chemtek Inc, Massachusetts, USA), ethyl acetate (Macklin Biochemical Co., Ltd, Shanghai, China), 2-cyclohexen-1-one (Macklin Biochemical Co., Ltd), n-hexane (Aladdin Bio-Chem Technology Co., LTD, Shanghai, China), glucose (Sinopharm Chemical Reagent Co., Ltd, Beijing, China), and proline (Wako Pure Chemical Industries, Ltd, Chuo-ku, Japan) were prepared. SPME fiber coatings used in this project were 1cm long divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μ m), PDMS/DVB (65μm), CAR/PDMS (85 μm), PDMS (100 μm), and polyacrylate (PA, 85 μm) (Supelco, St. Louis, MO, USA).

2.2. Preparation of raw and baked red jujube samples

Red jujube samples were bought from three major red jujube trading markets (Beiyuanchun Jujube Market in Xinjiang province, Cuierzhaung Jujube Market in Hebei province, Mengzhuang Jujube Market in Henan province) in China. Five cultivars of red jujube (Junzao, Huizao, Jinsixiaozao, Lingwuchangzao, and Minghshandazao) were collected 50 kg separately and stored at 4 °C until tested (Table 6-1). Red jujube (100 g) of each cultivar without pests and diseases were cleaned, kernel removed, frozen by liquid nitrogen and ground into fine pulp with Joyoung grinder (JYL-C93T, Shandong, China). No DDMP was detected in the raw red jujube, thus it was used as a DDMP-free matrix. For the preparation of baked red jujube samples, red jujube (500 g) of each cultivar without pests and diseases were cleaned, kernel removed, sliced (4 mm) and put into an ovenware tray and baked at 180 °C for 4 min in an Isotemp forced-air oven (PT 2531, Media Co., Ltd., Anhui, China). The baked slices were frozen by liquid nitrogen and ground into fine powder immediately. Accurate 1.00 ± 0.02 g of powder was put into a 20 mL vial (Welch Materials,Inc., Maryland, USA) sealed with a magnetic screw-cap and septum (Welch) before testing.

Table 6-1 Information of five cultivars of raw and baked red jujubes.

Code	Cultivar	Mean annual pluviometry(mm)	Mean annual temperature (°C)	Mean relative humidity (%)	Originate	Raw samples	Baked samples
JZ	Junzao	70	5.7	56	Xinjiang Province, Akesu City		
HZ	Huizao	70	5.7	56	Xinjiang Province, Akesu City		
JSXZ	Jinsixiaozao	600	13.3	58	Hebei Province, Cangzhou City		
LWCZ	Lingwuchangzao	445	8.4	60	Ningxia Province, Lingwu City		
MSDZ	Mingshandazao	88	9.5	57	Gansu Province,Dunhuang		

City

The mean annual pluviometry was estimated over the previous 3 years.

The information of mean annual pluviometry, mean relative humidity and temperature was achieved from CHINA METEOROLOGICAL DATA SERVICE CENTRE National Meteorological Information Centre (http://data.cma.cn/en).

2.3 Preparation of standard solutions

A volume of 10 μL 2-cyclohexen-1-one diluted in n-hexane was prepared as internal standard (IS) (10 μg/mL). Standard solutions of DDMP at different concentrations (0.01, 0.1, 1, 10, and 100 μg/mL) were obtained by dilution with ethyl acetate and being added into the DDMP-free jujube matrix to create a standard curve for quantitation. The standard solutions were stored at 4 °C and protected from light before testing within 24h.

2.4. Automated HS-SPME conditions

An automated SPME utilizing a PAL RSI 120 autosampler (CTC Analytics AG, Zwingen, Switzerland) was used in the project. Five kinds of SPME fiber coatings including DVB/CAR/PDMS, PDMS/DVB, CAR/PDMS, PDMS, and PA were tested for their abilities to extract DDMP from baked Junzao. The fibers were preconditioned at 250 °C for 30 min firstly. The fibers were compared by using an extraction temperature of 50 °C for 30 min, after a 40 min equilibration period with an agitation speed of 450 rpm, together with a weight of 1.00 g baked Junzao using as the test material. DVB/CAR/PDMS was selected as it provided the largest peak area of DDMP.

The DVB/CAR/PDMS extraction was further optimized by testing different extraction temperatures (30, 50, and 70 °C) and times (5, 15, 25, and 50 min) using 40 min equilibration time. The final optimized extraction conditions were 70° C for 25 min. The fiber was then desorbed for 8 min at 250 °C in the injection port of the GC equipped with a straight SPME inlet liner (0.75 mm ID, Agilent Technologies, Santa Clara, CA, USA).

2.5. GC-MS/MS system and operating conditions

GC-MS/MS analysis was performed on a Xevo TQ-GC GC-MS/MS system from Waters (Milford, USA) coupled with automated SPME. The chromatographic column was a DB-WAX (30 m × 0.25 mm × 0.25 μm, Agilent Technologies). Helium (99.999%) was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed with an initial temperature of 40 °C for 3 min, followed by a temperature ramp of 5 °C/min to 120 °C, 10 °C/min to 200 °C, with a final hold for 5 min. The samples were run in splitless mode with the split vent valve opened at 1.1 min with a flow of 30 mL/min and held until 2 min when it was reduced to 3 mL/min for the rest of the run. The MS transfer line and ion source were set at 250 °C and 200 °C, respectively. Instrument control, data acquisition, and processing were performed with MassLynx software (Waters).

2.6. Optimization of multiple reaction monitoring (MRM) conditions

A full mass scan mode (50-500 m/z) was conducted with standard solutions of DDMP (100 μg/mL) and IS (10 μg/mL) to select target precursor ions. Mass spectra were acquired in an electron impact mode at an ionization energy of 70 eV. Then, a daughter ion scan mode was conducted to get target daughter ions with a collision energy of 20eV. At last, the selected target ions were tested at various collision energy voltages (5, 10, 13, 15, 18, 20, 23, 25, 28, 30, 33, 35, 40, 45, 50 eV) to obtain the optimum MRM transitions. Argon gas (99.999% purity) was set at a flow rate of 1.5 mL/min and used as collision gas for ion fragmentation. Dwell times were set to ensure 15-20 cycles over a peak. Optimized MRMs were selected based on the highest mass counts closest to the precursor ions with minimal interferences. The following transitions were selected for DDMP: 144.10 → 54.60 (quantifier transition) and 144.10 →100.90 (qualifier transition); for IS: 96.00 → 68.00 (quantifier transition) and 96.00 → 40.00 (qualifier transition).

2.7. Validation of HS-SPME-GC-MS/MS method

The method was validated in terms of specificity, linearity, sensitivity, accuracy, and precision. To investigate the specificity of the method, the chromatogram of DDMP in baked Junzao was compared with the chromatogram of DDMP-free Junzao matrix (raw Junzao) to exclude the coelution of any endogenous interfering compounds at or close to the retention time of DDMP and IS. The evaluation of linearity, range, and linear regression function was performed by analyzing calibration curves of DDMP in concentrations of 0.01, 0.1, 1, 10 and 100 μg/mL (n=3). The LOD (signal-to-noise (S/N) of 3:1) was used to determine the sensitivity. The limit of quantitation (LOQ) (S/N of 10:1) was determined experimentally to be the lowest spiked concentration that most consistently gave an accuracy of 100 (± 15)%. Accuracy and precision were evaluated via the analysis of spiked samples using concentrations of 10, 20, 50 μg/mL (10 μL) for baked Junzao and baked Huizao. Repeatability was expressed as the relative standard deviation (RSD) of the calculated concentrations of DDMP. The accuracy of the method was expressed as bias calculated from the tests.

2.8. Determination of proline

The determination of proline was performed according to Cho et al (Cho et al., 2006). A Hitachi model L-8900 amino acid analyzer (Hitachi Co. Ltd., Tokyo, Japan) with a column packed with Hitachi custom ion-exchange resin 2622 (4.6 mm × 60 mm, particle size 5 μm) was applied for the analysis of amino acids. Accurate 2.0 g samples were macerated in 1% sulfo salicylic acid (50 mL) then ultrasonic treated for 20 min. A filtration (0.22 μm) volume of 20 μL was tested. Flow rates of lithium citrate buffer and ninhydrin was 0.35 mL/min and 0.30 mL/min, respectively. The column temperature ramped up from 30 °C to 70 °C and the reaction coil temperature was 135 °C.

2.9. Determination of glucose

Quantitative analysis of glucose in red jujubes was performed by high-performance anion-exchange chromatography equipped with pulsed amperometric detection (HPAEC-PAD) according to Šimkovic et al (2009). About 2.0 g homogenized red jujubes pulp was extracted with 50 mL water in ultrasonic bath (40 kHz) for 30 min. The extraction was carried out after centrifugation (20 min, 12,000 g). Then the filtered (0.45 μm) supernatant was analyzed in Dionex ICS-

3000 system. An injection of 10 μL was tested in a Carbo Pac™PA20 column (3 × 150 mm) with A (water) and B (250 mmol/L NaOH) as the mobile phase. The flow rate was 0.4 mL/min and gradient elution condition was as follows: 0-20 min, 95% A and 5% B; 20-30 min, 80% A and 20% B; 30-40 min, 0% A and 100% B; 40-50 min, 95% A and 5% B. The standards of glucose were tested at the same condition for quantitative analysis.

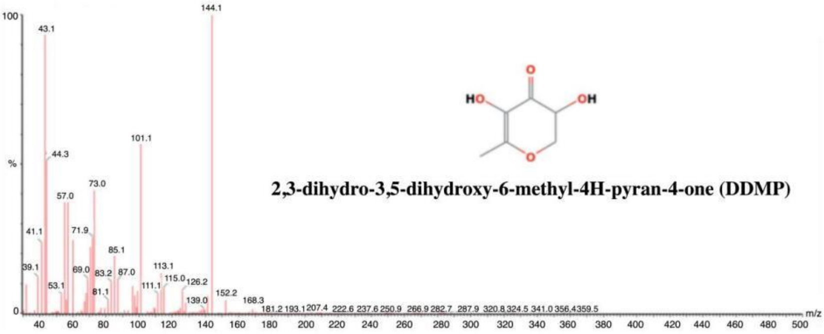
2.10. Data analysis

SPSS 21.0 Statistics (SPSS Inc.,Chicago, USA) was used for the analysis of variance (ANOVA) and multiple comparisons. The differences at $p < 0.05$ were considered significant. ClustVis was applied for heatmap analysis. R language (version 2.13.0) was used for the correlation analysis.

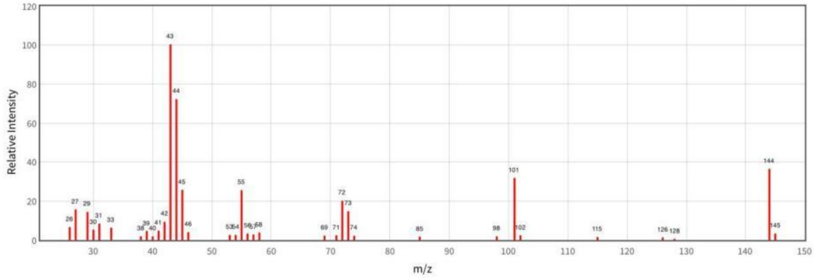
3. Results and discussion

3.1. Optimization of MRM conditions

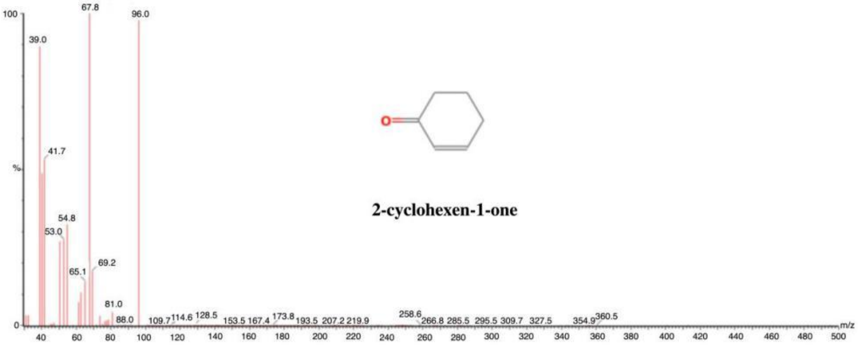
The precursor ion of DDMP and IS was *m/z* 144.10 and *m/z* 96.00, respectively (Figure 6-1). As was shown in Figure 6-2, quantifier transition and qualifier transition for DDMP were *m/z* 144.10 → 54.60 and *m/z* 144.10 → 100.90, respectively. Quantifier transition and qualifier transition for IS were *m/z* 96.00 → 68.00 and *m/z* 96.00 → 40.00, respectively. The collision energy and peak area for DDMP and IS were shown in Figure 6-3. The optimized collision energies for quantifier transition and qualifier transition were listed in Table 6-2. The peak area of *m/z* 144.10 → 100.90 and *m/z* 144.10 → 54.60 were the first and second largest when the collision energy were 20 eV and 13 eV, respectively (Figure 6-3). Besides, the collision energies of quantifier transition and qualifier transition for IS were both 20 eV (Figure 6-3).



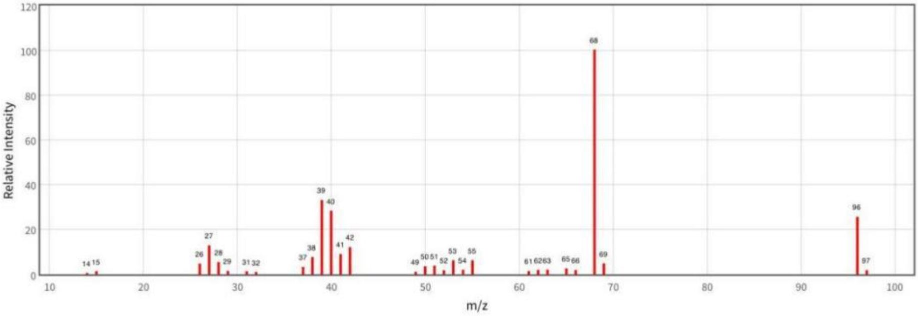
（a1）Mass spectrum of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)



(a2) Mass spectrum of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) cited from NIST Library

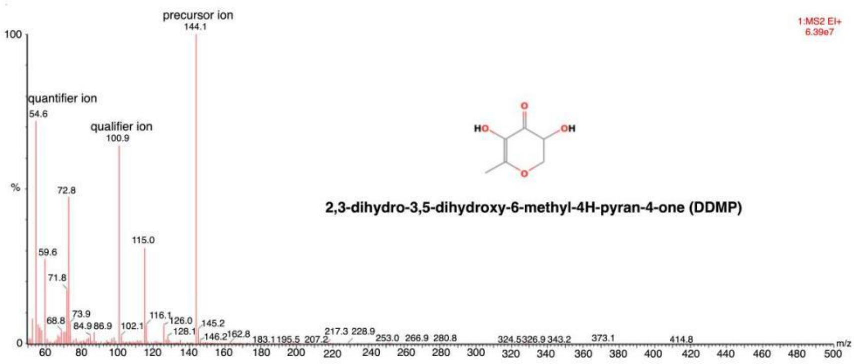


（b1）Mass spectrum of 2-cyclohexen-1-one

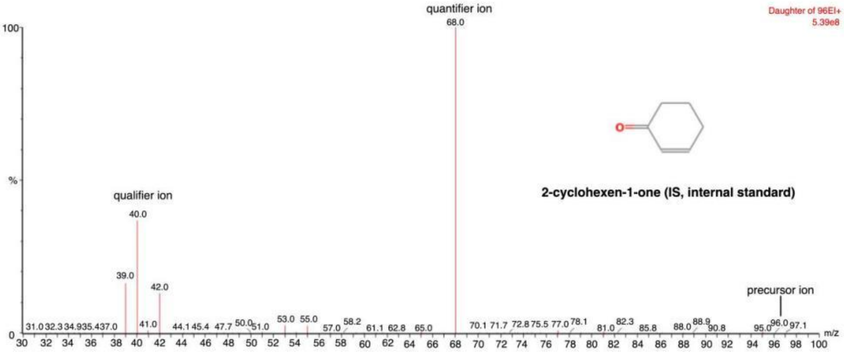


(b2) Mass spectrum of 2-cyclohexen-1-one cited from NIST Library

Figure 6-1 (a1) Electron ionization mass spectra for 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP); (a2) Mass spectrum of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) cited from NIST Library (https://webbook.NIST.gov/chemistry/name-ser/); (b1) Electron ionization mass spectra for 2-cyclohexen-1-one (IS: internal standard); (b2) Mass spectrum of 2-cyclohexen-1-one cited from NIST Library (https://webbook.NIST.gov/chemistry/name-ser/)



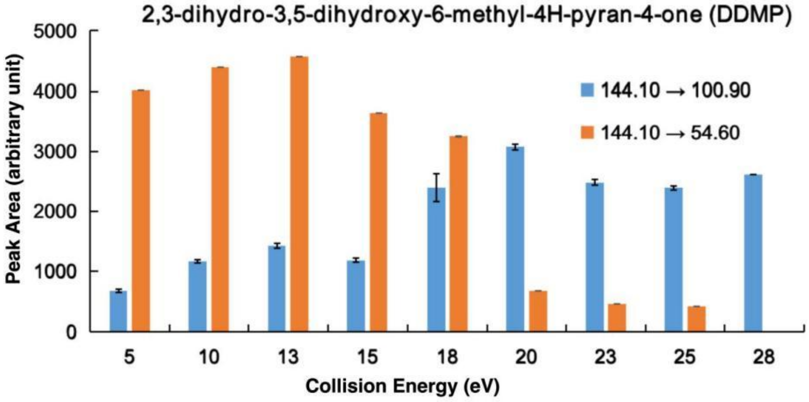
(a) Daughter ions of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)



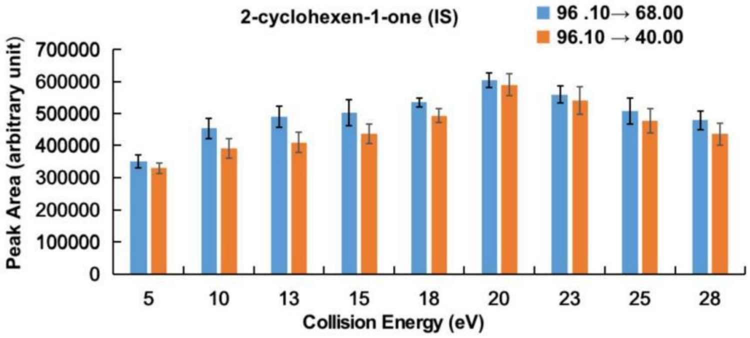
(b) Daughter ion of internal standard (IS, 2-cyclohexen-1-one)

Figure 6-2 Daughter ions of (a) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) in a daughter ion scan mode with precursor ion of *m/z* 144.1; (b) 2-

cyclohexen-1-one (IS, internal standard) in a daughter ion scan mode with precursor ion of *m/z* 96.



(a) The peak area and collision energy for 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one（DDMP）



(b) The peak area and collision energy for 2-cyclohexen-1-one (IS)

Figure 6-3 The optimization of collision energy for 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) and 2-cyclohexen-1-one (IS). DDMP was not detected when the collision energy was over 28eV (n=3).

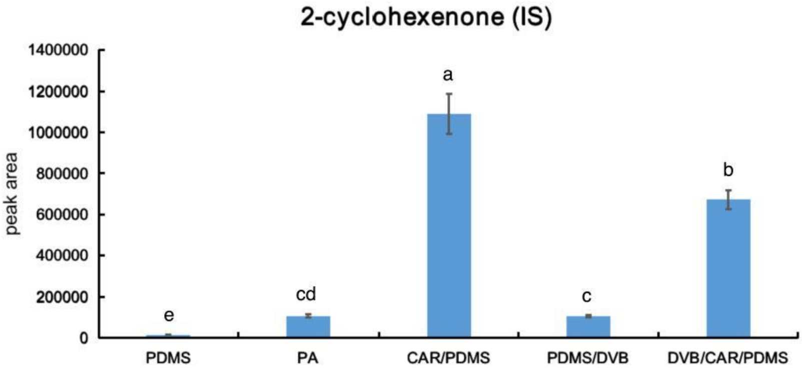
Table 6-2 The optimized condition of multiple reaction monitoring (MRM) mode for DDMP and IS.

compound	precursor ion	quantitative ion	collision energy (eV)	qualitative ion (eV)	collision energy	ratio* (%)
DDMP**	144.1	54.6	13	100.9	20	58.14±0.23
IS***	96	68	20	40	20	38.43±0.52

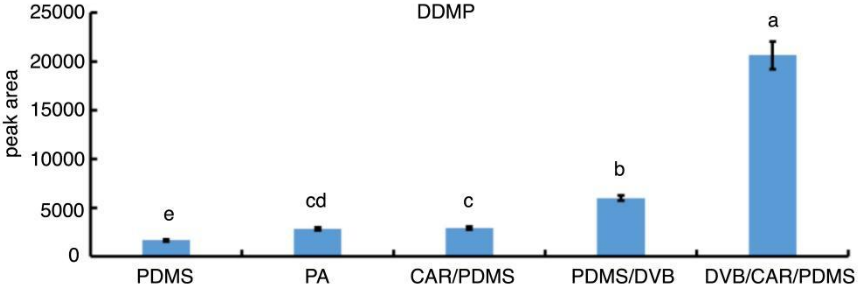
'*' represented the relative abundance ratio of qualitative ions to quantitative ions (n=3).
'**' DDMP:2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
'***' IS (Internal Standard):2-cyclohexen-1-one.

3.2. Optimization of HS-SPME conditions

DDMP extraction was optimized to ensure high-throughput analysis of DDMP at trace levels (far below the odor threshold of 2.06 μg/g) (Li et al., 2021b).The HS-SPME condition of fiber coatings was optimized at extraction temperature of 50 ℃ for 30 min after a 40 min equilibration period with an agitation speed of 450 rpm. As was shown in Figure 6-4, the peak area of DDMP reached the largest when DVB/CAR/PDMS was used. The peak area of 2-cyclohexen-1-one was the largest when CAR/PDMS was used, while the signal obtained with DVB/CAR/PDMS was roughly 38% lower, which was considered acceptable for the IS. Therefore, DVB/CAR/PDMS was selected as the fiber in this study. The result was consistent with that of Pereira et al., who also used DVB/CAR/PDMS to extract DDMP from coffee (Pereira et al., 2019).



(a) Peak area of 2-cyclohexen-1-one under different kinds of extraction fibers



(b) Peak area of DDMP under different kinds of extraction fibers

Figure 6-4 Comparison of five kinds of SPME fiber coatings for the extraction of (a) 2-cyclohexen-1-one (IS) and (b) DDMP from baked Junzao. The experiments were conducted at an extraction temperature of 50 ℃ for 30 min after a 40 min equilibration period using DB-WAX column. The sample was a weight of 1.00± 0.02 g baked Junzao with addition of 2-cyclohexen-1-one (10 μg/mL,10 μL) as internal standard (IS). (DDMP, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; CAR, carboxen; PDMS, polydimethylsiloxane; DVB, divinylbenzene; PA, polyacrylate)

The adsorption time and temperature were optimized to ensure the required sensitivity and a fast overall analysis of DDMP in baked Junzao (Figure 6-5). A volume of 10 μL IS (10 μg/mL) was added in the baked Junzao. Three extraction temperatures (30 °C, 50 °C and 70 °C) were tested, as well as four extraction times for each temperature (5, 15, 25, and 40 min). Clearly, the extraction efficiency of DDMP increased by raising the temperature to 70 °C. When the adsorption temperature was 30 °C and 50 °C, the area of IS did not change much. However, when the extraction temperature was raised to 70 °C, the area of IS increased a little. Thus the adsorption efficiency of both DDMP and IS was higher when the extraction temperature was 70 °C. Under the extraction temperature of 70 °C, it was observed that the area of DDMP increased gradually when the extraction time was extended from 5 min to 25 min. However, when it was extended to 40 min, the extraction efficiency was not significantly improved. For IS, extraction time has no significant effect on extraction efficiency. Therefore, the optimal extraction condition could be determined as 70 °C for 25 min.

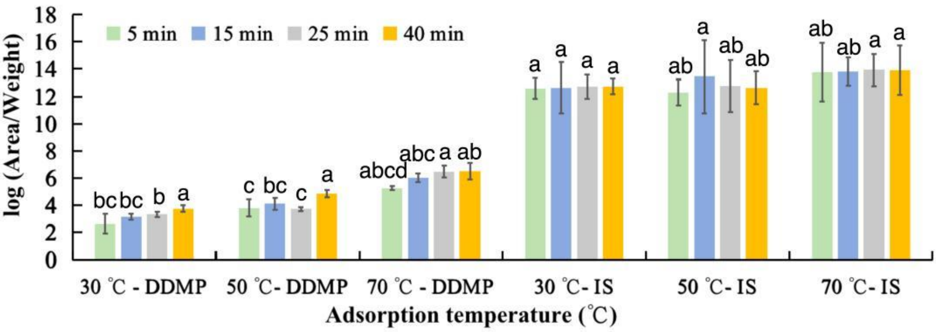


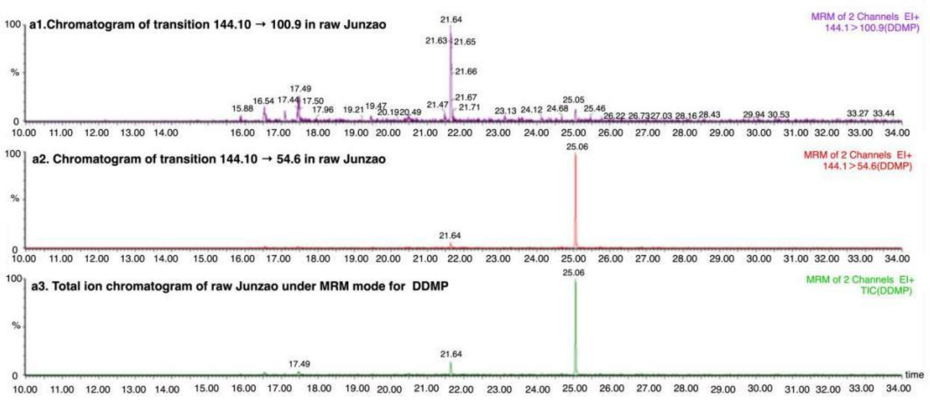
Figure 6-5 Comparison of extraction time (5, 15, 25, 40 min) and temperature (30, 50, 70 °C) for DDMP and the IS. Error bars are standard deviations (n = 3). A weight of 1.00±0.02 g baked Junzao with addition of 10 μL IS (10 μg/mL) was used as the tested sample (DDMP, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; IS (Internal Standard), 2-cyclohexen-1-one). Different letters indicate significant differences at $p < 0.05$.

3.3. Validation of HS-SPME-GC-MS/MS method

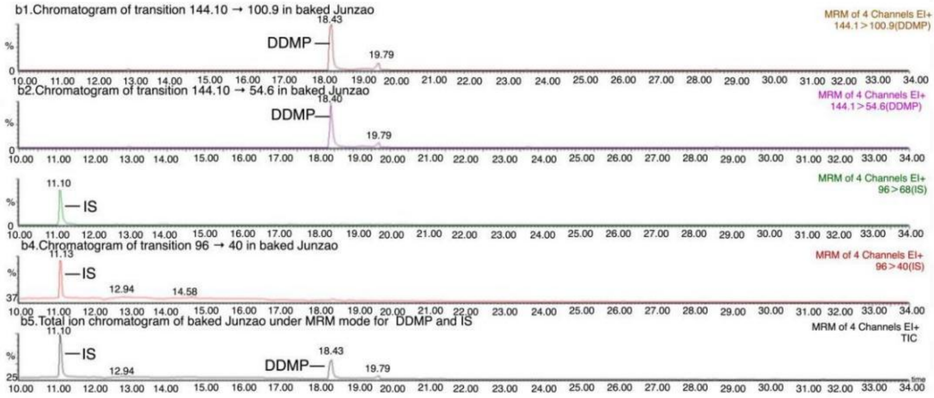
The target compound was identified according to European Commission (SANTE/11813/2017) rules. The information of retention time, mass-to-charge ratio, and monitor ion to peak area ratio should be examined. The retention time shift of the target compound in the sample and the standard should be within ±0.1 min. The mass-to-charge ratio of the target compound should be compared with that from references. For the identification of the target compound, the peaks of the selected ions should overlap completely. Moreover, the peak area ratio is prescribed into four levels: >50%, 20–50%, 10–20% and >10%, in corresponding to the deviation of ±20%, ±25%, ±30% and ±50%, to demonstrate the reliability of the method (Chinese Pharmacopoeia (2015 Edition)). The method was validated with regard to precision, accuracy, specificity, linearity, LOD, and LOQ.

3.3.1 Specificity

As was shown in Figure 6-6 (a), there were no peaks in raw Junzao around retention time of 18.40 min when the transition were *m/z* 144.10 → 54.60 and *m/z* 144.10 → 100.90. In Figure 6-6 (b), the peaks were sharp and high in baked Junzao around a retention time of 18.40 min when the transitions were *m/z* 144.10 → 54.60 and *m/z* 144.10 → 100.90. Thus the specificity was proved that there were no peaks around the peak for DDMP in raw Junzao compared to baked Junzao. Besides, chromatograms showed the retention time of IS was 11.10 min. The retention time shifts for DDMP and IS were 0.02% and 0.03%, respectively (n=3). The mass spectra of DDMP and IS were compared with those in the NIST library (Figure 6-1). DDMP and IS were tentatively identified. According to Table 6-3, the retention time of DDMP in baked red jujubes was within ±0.1 min compared to the standard. The peak area ratio of the DDMP standard was 58.14%. The deviation of the ratio in baked red jujube samples and the DDMP standard was in the 5.65-17.05% range, lower than the 20% maximum variation required by European Commission (SANTE/11813/2017) rules.



(a) Chromatograph of raw Junzao under MRM mode for 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)
(n=3)



(b) Chromatograph of baked Junzao under MRM mode for 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) and internal standard (IS, 2-cyclohexen-1-one) (n=3)

Figure 6-6 The chromatograph of the test for 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) in (a) raw Junzao compared to (b) baked red Junzao

Table 6-3 The monitor ion to peak area ratio of DDMP in baked red jujube and the standard.

	DDMP Standard (n=3)	baked Huizao (n=5)	baked Junzao (n=5)	baked Jinsixiaozao (n=5)	baked Lingwuchangzao (n=5)	baked Mingshandazao (n=5)
ratio * (%)	58.14±0.23	48.46±11.96	64.75±7.70	52.49±2.62	65.22±10.46	41.09±0.37
deviation **(%)	/	9.68	6.61	5.65	7.08	17.05
retention time shift (min)	0.01	0.04	0.01	0.04	0.01	0.03

‘*’represented the monitor ion to peak area ratio of qualitative ions to quantitative ions.

‘**’ represented the deviation between the ratio* of DDMP in baked red jujube to that in the standard.

DDMP: 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.

3.3.2 Linearity and sensitivity

The evaluation of linearity and range was performed by analyzing the matrix-matched calibration curve in the ranges of 10-100000 ng/g for baked Junzao, using DDMP-free raw Junzao as calibration medium (R^2 = 0.992). The LOD was determined with the concentration of 0.1 ng/g (144.10 → 54.60, S/N=10.26 > 3; 144.10 → 100.90, S/N= 5.96 > 3), which was lower than the threshold of DDMP (2.06 μg/g). Whereas, the LOQ was determined to be the concentration of 0.3 ng/g (144.10 → 54.60, S/N=22.15 > 10; 144.10 → 100.90, S/N= 12.87 > 10).

3.3.3 Accuracy and precision

Intraday and interday precision and accuracy of DDMP was shown in Table 6- 4. Three different concentrations (10, 20, and 50 μg/mL) of DDMP (10 μL) was added in baked Junzao and baked Huizao to evaluate precision by RSD (%), and to evaluate the accuracy expressed as bias. RSD and recovery were demonstrated by the seven consecutive repeated analyses of standard solutions at 10, 20, and 50 μg/mL on the same day and by the repeated analysis of a sample for four consecutive days (three repeated analyses each day), respectively. The RSD of the intraday and interday repeated analysis were ≤ 7% and ≤ 11% in baked Junzao, and the bias of DDMP ranged from 1.2% to 12.1%. At the same time, the RSD of the intraday and interday repeated analysis were ≤ 10% and ≤ 14% in baked Huizao, and the bias of DDMP ranged from 3.8 % to 14.6 %. In general, the results indicate a reasonable precision and accuracy of the method for the determination of DDMP in baked red jujube.

Table 6-4 Precision and accuracy of DDMP obtained by HS/SPME GC-MS/MS

Cultivar	Concentration (μg/mL)	Intraday*		Interday**	
		bias (%)	RSD (%)	bias (%)	RSD (%)
Baked Junzao	10	5.4	6.9	8.5	10.3
	20	3.2	4.3	12.1	9.2
	50	11.4	5.4	1.2	3.6
Baked Huizao	10	4.3	3.8	4.3	4.2
	20	7.5	2.5	3.8	5.9
	50	6.3	9.3	14.6	13.4

‘*’The intraday experiment was conducted with standard solutions and baked Junzao and Huizao samples by seven times repeated analysis at the same day (n=7).

‘**’The interday experiment was conducted with standard solutions and baked Junzao and Huizao samples by three times repeated analysis in four different days (n=12).

DDMP: 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.

3.4. Effect of cultivars on DDMP contents in baked red jujube

The optimized and validated method was used to evaluate the amount of DDMP in five cultivars of baked red jujube, as well as to evaluate the effect of geographical differences. The content of DDMP in baked red jujubes varied from 4.55 µg/g to 46.50 µg/g (Figure 6-7). The content of DDMP was the highest in baked Mingshandazao (46.50 µg/g), followed by baked Junzao (34.15 µg/g), Lingwuchangzao (5.96 µg/g), Jinsixiaozao (5.61 µg/g), and Huizao (4.55 µg/g) in decreasing order. The content of DDMP in baked Mingshandazao and Junzao was about ten folds higher than that of baked Lingwuchangzao, Jinsixiaozao and Huizao.

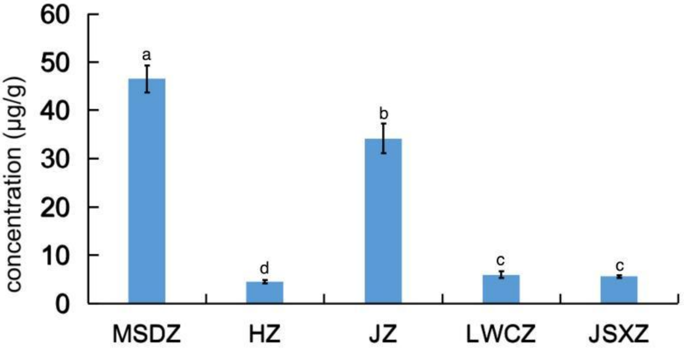


Figure 6-7 The content of DDMP in different cultivars of baked red jujube (n=5)

(DDMP, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; MSDZ: Mingshanzazao; HZ: Huizao; JZ: Junzao; LWCZ: Lingwuchangzao; JSXZ: Jinsixiaozao)

3.5. Analysis of different DDMP content in baked red jujubes

The formation of DDMP is related to the Maillard reaction, which is mainly affected by temperature, water content, sugar, amino acid and other precursors (Li et al., 2019a). DDMP can be formed through two formation pathways, namely by 2,3-enolization pathway of the Maillard reaction, and on the other hand, it can also be formed during the heating of hexose (Nishibori et al., 1994; Shaw et al., 1971). The research about factors on the formation of DDMP in a solid-state Maillard reaction system demonstrated that the proline and reactant molar ratio of proline to glucose have a key effect on the formation of DDMP. When the ratio of proline to glucose is greater than 1, especially when it reaches 2, the content of DDMP formed in the system is the highest (Zhou et al., 2014). Thus, the different content of glucose and proline in the five cultivars of red jujubes may also correlate to the difference in DDMP content in baked red jujubes. As was shown in Table 6-5, the content of proline in raw Mingshandazao was the highest (3.26 mg/g), followed by raw Junzao (2.26 mg/g), raw Huizao (1.66 mg/g), raw Lingwuchangzao (1.21 mg/g) and raw Jinsixiaozao (1.02 mg/g). Correlation analysis was carried out to clarify the relationship between the contents of DDMP and proline, glucose, moisture content, and resist water stress (Figure 6-8). The contents of DDMP in red jujube were positively correlated with proline and the molar ratio of proline to glucose. Therefore, the higher proline content and reactant molar ratio of proline to glucose in Mingshandazao and Junzao might be the reason for the higher DDMP content in the two cultivars of baked red jujubes.

As for the factors affecting Maillard reaction, reaction phase, amino acid properties and moisture content has been proved to affect the generation of DDMP. Moisture content can change the enolization site in the glucose-proline system, which could affect the formation of DDMP. A previous research demonstrated that the lower moisture content facilitates the formation of DDMP in the glucose-proline system (Li et al., 2019a). As was shown in Table 6-5, the moisture content in MSDZ (22.52%) and JZ (20.44%) was about 10% lower than that of HZ (31.56%), JSXZ (30.55%) and LWCZ (35.83%). Besides, the contents of DDMP in red jujube were negatively correlated with moisture content (Figure 6-8). Thus, the lower moisture content in MSDZ and JZ could also promote the formation of DDMP which might lead to a higher content of DDMP.

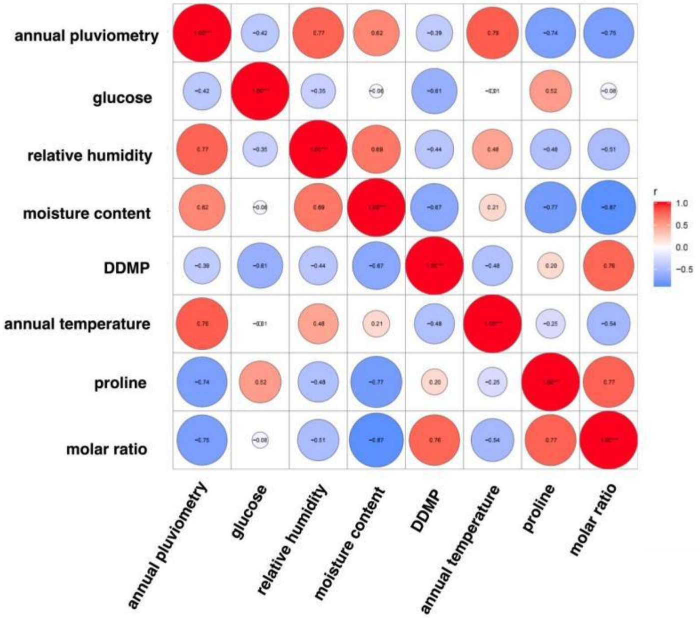


Figure 6-8 Correlation analysis between the contents of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) and glucose, proline, moisture content and resist water stress.

Besides the correlation with the Maillard reaction described above and genetic influence on the DDMP content of baked red jujube, the environmental factors in the cultivation process also have an influence on the DDMP content. In fact, the cultivation conditions may affect the accumulation and composition of chemicals like sugars and amino acids, and thus affect the formation of DDMP.

The biosynthesis of proline in plants occurs mainly from glutamate via two NADPH-dependent enzymes, which were encoded by two genes (Furlan et al., 2020). Studies have shown that the stress tolerance of plants, which is decided by genes, cultivation practices, or environmental factors, is closely related to the accumulation of endogenous proline (Ben Hassine et al., 2008; Evers et al., 2010). For example, a higher accumulation of proline was observed in the drought-stress tolerant cultivar of peanuts (Ranganayakulu et al., 2015). Jujube, as an extremophile, has an excellent capability to survive under arid conditions with limited water availability, exhibiting high drought stress tolerant ability (Kumar et al., 2020). The plant tolerance to drought stress can also vary substantially between cultivars (Furlan et al., 2010).Therefore, the gene-determined drought stress tolerant ability between cultivars will indirectly lead to the accumulation of proline content in red jujube. As was shown in Figure 6-8, the relationship between DDMP and annual pluviometry, annual temperature, and relative humidity was negative. Thus, red jujube cultivars with a stronger ability to resist water stress would be more likely to produce DDMP during the baking process. According to Table 6-1 and Table 6-5, the lower mean annual pluviometry and moisture content of Junzao and

Mingshandazao reflected the fact that they might suffer from stronger water stress than others. Therefore, the two drought-adapted red jujube cultivars (Junzao and Mingshandazao) might also produce more proline in response to water stress than the other cultivars, which leads to a higher accumulation of DDMP.

Table 6-5 The contents of glucose, proline and moisture content in Huizao, Junzao, Jinsixiaozao, Lingwuchangzao and Mingshandazao

Indicators	Huizao	Junzao	Jinsixiaozao	Lingwuchangzao	Mingshandazao
glucose (mg/g)	96.93±7.83a	55.25±4.56c	70.27±0.06b	62.44±10.23b	101.44±10.23a
proline (mg/g)	1.66±0.12c	2.17±0.12b	1.02±0.05e	1.21±0.13d	3.26±0.20a
reactant molar ratio of proline to glucose	0.03±0.002c	0.06±0.003a	0.02±0.0012d	0.03±0.0023c	0.05±0.0021b
moisture content (g/100g)	31.56±2.21b	20.44±1.02d	30.55±4.23b	35.83±1.23a	22.52±1.40c

The results were expressed as mean ± SD (standard deviation) (n = 3). Different letters indicate significant differences at $p < 0.05$.

Besides, taking into the factors (moisture content, glucose, proline and the molar ratio of proline to glucose) that affect the formation of DDMP in baked red jujube, the heatmap analysis showed that the cultivars of Huizao, Jinsixiaozao, and Mingshandazao clustered together except for Junzao and Mingshandazao (Figure 6-9). Thus, the cluster analysis also proved the reliability of the inference that DDMP was closely related to those chemical compositions in the correlation analysis.

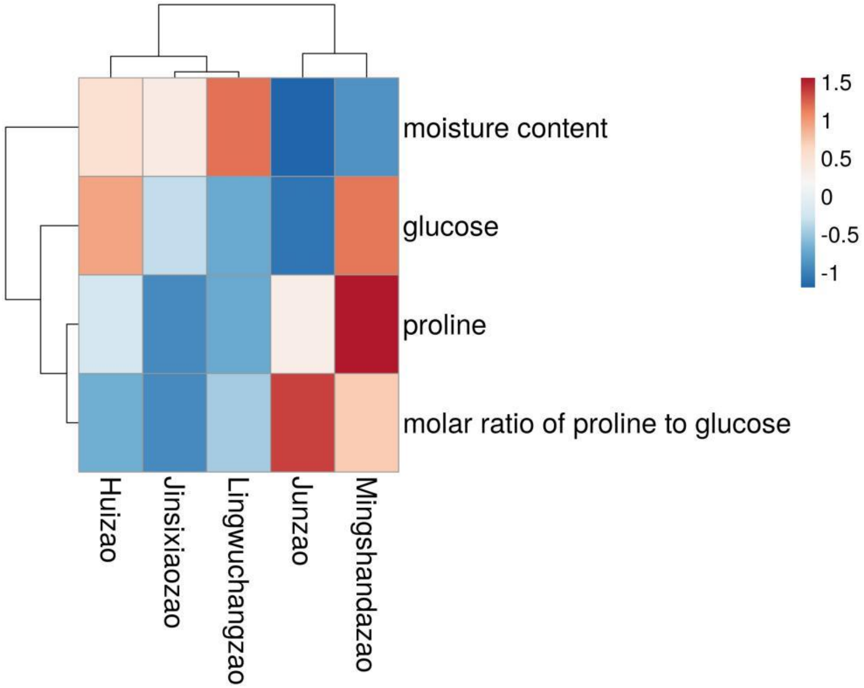


Figure 6- 8 Heatmap analysis of cultivars of Mingshandazao, Junzao, Lingwuchangzao, Jinsixiaozao, and Huizao based on chemical compositions.

As one of the substances originated from the Maillard reaction, DDMP could generate active oxygen species to cause DNA strand breaking and mutagenesis in Salmonella typhimurium TA100 without metabolic activation (Kazuyuki et al., 1997). DDMP could also affect sympathetic nerve activity and metabolism in rats though the mechanism was not determined (Beppu et al., 2012). On the other hand, DDMP was proved to inhibit the activity of tyrosinase which could prevent mushrooms from enzymatic browning (Xu, Zhang, &Karangwa, 2016). DDMP from onions could also inhibit colon cancer cell growth by inducing apoptotic cell death through the inhibition of NF-KB (Jung et al., 2007). Besides, the DDMP moiety can attach to the triterpene aglycone making it possible for scavenging toward active oxygen in soybean (Yoshiki & Okubo, 1995). The studies demonstrated that as one of the Maillard reaction products, DDMP contributed to the antioxidant activity and might present a synergistic effect with other Maillard reaction products (Yu et al., 2013). It was also speculated that the exhibited antioxidant capacities of DDMP were in relation to its stabilized endiol structure incorporated in the corresponding heterocycles both under laboratory conditions and in food (Kanzler et al., 2016).

As one of the heterocyclic compounds presented in thermal-treated foods and beverages, the qualitative and quantitative analysis set up in this project for the determination of DDMP is of high importance to evaluate food processing and storage protocols in practical applications.

4. Conclusions

In brief, a method for the quantitation of DDMP in baked red jujubes using HS-SPME GC-MS/MS has been developed. The method was validated and was able to determine the DDMP content in cultivars of baked red jujube reliably and sensitively, in a range between 0.01-100 μg/g. Information on the occurrence of DDMP in red jujube was recorded and correlation with cultivars and geographical origin was examined. The contents of proline, glucose and moisture content in cultivars of red jujube, as well as the ability to resist water stress related to the formation of proline, might correlate with the formation of DDMP.

7

ChapterⅦ. HS-SPME-GC-MS/MS Method for Investigation of Effects of Baking on Distribution of Four Alkyl Pyrazines in Red Jujube

Alkyl-substituted pyrazines that occurred in raw, roasted, baked or other cooked foods were perceived as nutty, roasted, musty or sweet odors with low odor threshold, eg., ng/L, even pg/L, which are highly appreciated by people. Kinds of pyrazines have been determined in baked red jujube, such as 2-methyl pyrazine, 2,6-dimethylpyrazine, and trimethylpyrazine, which contributed to toasty, roasty, and nutty odor characteristics, separately. The main objectives of this study were to develop an analytical method with high sensitivity and accuracy for the quantitation of four alkyl-substituted pyrazines (methylpyrazine, 2,6-dimethyl pyrazine, trimethylpyrazine, and tetramethylpyrazine) in raw and baked red jujube.

This article has not been published yet.

Abstract

Alkyl pyrazines, playing an important role in flavor, occurs naturally in plants or can be generated via the Maillard reaction. A reliable and rapid method using headspace solid-phase microextraction gas chromatography-tandem mass spectrometry (HS-SPME-GC-MS/MS) has been developed to characterize and quantify alkyl pyrazines in raw and baked red jujube. The limits of detection (LODs) and limits of quantitation (LOQs) for the pyrazines were in the range of 0.5–10 pg/g and 1.5–30 pg/g, respectively. Good linear correlation coefficients (0.9947–0.9988) were obtained over the concentration ranges of 1–10000 ng/mL. Trimethylpyrazine, which accounted for 38.71% ~ 59.50% of the total pyrazine, was the main alkyl pyrazine in raw red jujube. Methylpyrazine accounted for 35.52% ~ 49.76%, the highest portion of alkyl pyrazine in baked red jujube. The developed method can be extended to monitor the composition of alkyl pyrazine in red jujube-related foodstuff.

Keywords: Alkyl pyrazines, Baked red jujube, HS-SPME-GC-MS/MS, Quantification

1. Introduction

Red jujubes (*Ziziphus jujuba* Mill.) are consumed as delicious fruit and can be added to foods like cakes, bread, yogurt, tea, etc., to improve sensory, nutritional and beneficial health characteristics (Najjaa et al., 2020; Feng et al., 2019; Ahmed et al., 2020). Within the foodstuffs mentioned before, the addition of red jujube in sponge cake not only improved the functionality and the sensory properties but also presented satisfactory consumer acceptability (Najjaa et al., 2020). Baking temperature (160 °C~200 °C) is of vital importance in regards to the final aroma of cakes (Goh et al., 2019). Red jujube in sponge cakes which is are baked at high temperature (180 °C) gives a distinctive and evocative aroma (Najjaa et al., 2020).

Pyrazines are a class of compounds derived from amino acids and commonly distributed in nature, such as food and water. Their structure is characterized by a single aromatic ring containing two nitrogen atoms that can carry substituents on one or more carbon atoms of the four-ring carbon atoms (Muller & Rappert, 2010). Generally, alkyl-substituted pyrazines occurred in raw, roasted, baked or other cooked foods and were perceived as nutty, roasted, musty or sweet odors with low odor threshold, ie., ng/L, even pg/L (Wang et al., 2020; Javier & Maria, 2019). For example, methylpyrazine in perilla seed oils gives the sweet and roasted flavors, reaching a peak area ratio of 15.86% (Kim et al., 2000). As to the 2, 6-dimethylpyrazine identified in cheese, olfactory tests found that it caused roast nut flavor characteristics (Frank, Owen, & Patterson, 2004). Trimethylpyrazine and tetramethylpyrazine were sniffed as two of the most potent odorants in vinegar with roasted potato and peanut odor separately (Zhou et al., 2017). Furthermore, some of the alkyl-substituted pyrazines were reported to have biological activities. For instance, tetramethyl pyrazine showed the ability to improve antithrombogenicity on silk fibroin (Lian et al., 2008). Tetramethylpyrazine exhibits antioxidant and anti-inflammatory functions (Sue et al., 2009). On the other hand, some pyrazines have been determined in baked (90 °C, 110 °C, 130 °C, and 150 °C) red jujube, such as 2-methyl pyrazine, 2, 6-dimethylpyrazine, and trimethylpyrazine, which contributed to toasty, roasty, and nutty odor characteristics, respectively (Zhao, 2018). In addition, the oral LD50 (lethal dose of 50% of a group of test animals) of methyl pyrazine, 2,6 -methyl pyrazine, trimethyl pyrazine, and tetramethyl pyrazine in rat is 1800, 880, 806, and 1916 mg/kg body weight (Moran et al.,1980). The previous study determined the concentrations of the four alkyl pyrazines’ content as 90, 34, 38, and 7 μg/kg in Ziziphus jujuba Mill., cv. Youzao puree using HS-SPME GC-MS (Zhu & Xiao, 2018). A reference daily intake for cv. Youzao puree in terms of these four alkyl pyrazines could be calculated as 20 g/kg body weight. Head space solid-phase microextraction (HS-SPME) is widely applied for the extraction of pyrazines from varieties of matrix, including raw and baked red jujube, peanut, and seed oils (Zhao, 2018; Baker et al., 2003; Kwon, Park & Jung, 2013). The use of HS-SPME presents a series of advantages like labor and time saving, easy to operate, free from sample loss comparing to other techniques, eg., steam distillation, solvent extraction (Sabik, Fortin, & Martin, 2012). For the analysis of targeted compounds, GC-tandem mass spectrometry (MS/MS) in MRM mode, implements better selectivity through qualitative ions, attains higher sensitivity through quantitative ions, and attenuates matrix effect, advantaging over GC-MS and GC×GC-MS that are proficient at untargeted metabolomics profiling and prone to interference with certain masses in matrix (Zeki et al., 2020; Neda et al., 2021; Tsikas & Alexander, 2014). Thus HS-SPME-GC-MS/MS, as a combination of the previously mentioned techniques, could perform well for the fast, accurate and precise analysis of volatiles. By now, HS-SPME-GC-MS/MS has played a pivotal role in the identification and quantitation of 2 - acetyl-1-pyrroline in rice, chemical warfare agent in the environment, volatile and semi-volatile organic compounds in wastewater, and C8 volatiles in mushrooms (Hopfer et al., 2016; Nawała et al., 2016; Saber, Zhang & Yang, 2016; Jung et al., 2021). Pyrazines can form in either chemical (e.g. Maillard reaction) or natural ways (Zhao et al., 2020). Nevertheless, the targeted method for alkyl pyrazines' quantitation in raw and baked red jujube has not yet been established. Thus, it is necessary to set up a method for detecting of the four mentioned alkyl pyrazines' content and achieve a reference daily intake amount for raw and baked red jujube.

The main objectives of this study was to develop an analytical method with high sensitivity and accuracy for the quantitation of four alkyl-substituted pyrazines (methylpyrazine, 2,6-dimethyl pyrazine, trimethylpyrazine, and tetramethylpyrazine) in raw and baked red jujube firstly. Then, the method will be validated on accounts of specificity, linearity, and precision. Moreover, the effect of baking on the composition of these four pyrazines will be further investigated. This work can be extended to monitor the changes of pyrazines in red jujube-based foodstuffs.

2. Material and methods

2.1. Chemicals

Mehanol (≥ 99.99%) of GC grade was obtained from Meridian Medical Technologies, Inc., Columbia, USA. The standards of methylpyrazine (≥99%), 2,6-dimethylpyrazine, trimethyl pyrazine (≥99%), and tetramethylpyrazine (≥99%) were purchased from TCI Chemical Industry Development Co., Ltd. (Shanghai, China). 2 -Cyclohexen-1-one was obtained from Macklin Biochemical Co., Ltd, (Shanghai, China). The SPME fiber coatings used in this project were 1cm long divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μ m) (Sigma-Aldrich Co., Ltd., Shanghai, China), PDMS/DVB (65μm), CAR/PDMS (85 μm), PDMS (100 μm), and polyacrylate (PA, 85 μm) (Supelco, St. Louis, MO, USA).

2.2. Chemicals preparation

2-Cyclohexen-1-one diluted in methanol (10 μg/mL) was prepared as internal standard (IS). The mixed standard solutions of methylpyrazine, 2,6-dimethylpyrazine, trimethyl pyrazine, and tetramethylpyrazine at different concentrations (0.001 μg/mL, 0.01 μg/mL, 0.1 μg/mL, 1 μg/mL, and 10 μg/mL) were obtained by dilution with methanol. The standard solutions were stored at 4 °C in dark before testing within 24h. In order to consider the matrix effect, the standard solutions of pyrazines and IS were added into pyrazine-free red jujube matrix.

2.3. Preparation of red jujube samples

Red jujube samples were bought from three major red jujube trading markets (Beiyuanchun Jujube Market in Xinjiang province, Cuierzhaung Jujube Market in Hebei province, Mengzhuang Jujube Market in Henan province) in China. Five cultivars of red jujube (Junzao, JZ; Huizao, HZ; Jinsixiaozao, JSXZ; Lingwuchangzao, LWCZ; Minghshandazao, MSDZ) were collected 50 kg separately and stored at 4 °C until tested (Table 7-1). Red jujube (600 g) of each cultivar without pests and diseases were cleaned, kernel removed, and sliced into 4mm. Half of the slices were put into an ovenware tray and baked at 180 °C for 4 min, using an Isotemp forced-air oven (PT 2531, Media Co., Ltd., Anhui, China). The raw and baked red jujube slices were then frozen with liquid nitrogen and ground into fine pulp with a Joyoung grinder (JYL-C93T, Shandong, China). An accurate 2.00 ± 0.02 g of raw/baked red jujube with an addition of 10 μL IS was put into a 20 mL vial (Welch Materials, Inc., Maryland, USA) sealed with a magnetic screw-cap and septum (Welch) before testing.

Preparation of pyrazine-free red jujube matrix: firstly, the slices of 500 g red jujube were treated with 40 kHz ultrasonic bath for 2 h for five consecutive times; secondly, the jujube slices were soaked in methanol and pentane subsequently for 48h, and the residue was filtered and ventilated for drying; thirdly, the pre-dried jujube slices were pre-freezed at -40℃ for 3 h and freeze-dried by vacuum freeze dryer with cold trap temperature of -50 °C, sublimation temperature of 25 °C, and vacuum pressure of 0.01 kPa (Alpha 1-4 LD plus, Marin Christ, Osterode, Germany). The vacuum freeze drying continued for 36 h until the moisture content of JZ samples was below 7 g/100 g. Pyrazines could not be detected in the pyrazine free red jujube matrix under the optimized HS-SPME-GC-MS/MS method. The pyrazine -free red jujube matrix was prepared and stored at −40 °C.

Table 7-1 The information of five cultivars of raw and roasted red jujube samples in the experiment.

Name	Cultivar	Originate	Raw samples	Baked samples
JZ	Junzao	Xinjiang Province, Akesu City		
HZ	Huizao	Xinjiang Province, Akesu City		
JSXZ	Jinsixiaozao	Hebei Province, Cangzhou City		
LWCZ	Lingwuchangzao	Ningxia Province, Lingwu City		
MSDZ	Mingshandazao	Gansu Province, Dunhuang City		

2.4. Automated HS-SPME conditions

The HS-SPME step was accomplished by a PAL RSI 120 autosampler (CTC Analytics AG, Zwingen, Switzerland). The fibers of DVB/CAR/PDMS, PDMS/DVB, CAR/PDMS, PDMS, and PA were evaluated based on the peak area of alkyl pyrazines in baked JZ using the extraction temperature of 50 °C and extraction time of 40 min. The preconditioning of the five fibers was conducted at 250 °C for 30 min. For the optimization of extraction conditions, extraction temperatures of 30, 50, and 70 °C, and extraction times of 5, 15, 25 and 40 min were considered. An agitation speed of 300 rpm was used during the extraction. The optimized extraction condition was set based on the most intense largest peak area and the shortest extraction time of the four pyrazines and IS.

2.5. GC-MS/MS analysis

GC-MS/MS analysis was performed on a Xevo TQ GC-MS/MS system from Waters (Milford, USA) equipped with a straight SPME inlet liner (0.75 mm ID, Agilent Technologies, Santa Clara, CA, USA). The columns of DB-5 (30 m × 0.25

mm × 0.25 μm, Agilent Technologies) were tested based on the separation effect and peak area of pyrazines and IS, using helium (99.999%) as carrier gas (1.0 mL/min). The oven programmed temperature for column was as follows: started at 40 °C for 5 min, heated to 80 °C with a rate of 10 °C/min, then 100 °C at 3 °C/min, and to 120 °C with a ramp of 5 °C/min, at last arrived at 200 °C with a rate 10 °C/min and held for 5 min. The sample was desorbed in splitless mode, with the split vent valve opened at 1.1 min with a flow of 30 mL/min and held until 2 min. Then the valve was reduced to 3 mL/min for the rest of the run. The temperatures of the injection port, transfer line, and ion source were 280, 250, and 200 °C, respectively. Helium (99.99%) was used as quench gas at a flow rate of 2.25 mL/min. Argon gas (99.999% purity) was set at a flow rate of 1.5 mL/min and used as collision gas for ion fragmentation. MassLynx was applied for instrument control, data acquisition, and data processing.

2.6. Optimization of MRM condition

The qualification analysis of pyrazines in baked JZ and IS was finished by retention time and mass spectrum of the mixed standards of pyrazines (0.1, 1, and 10 μg/mL) and the standard of IS (10 μg/mL) in full mass scan mode (*m/z* 35–500) with ionization energy of 70 eV. The targeted precursor ions of pyrazines and IS was thus achieved. The targeted daughter ions were selected under a daughter ion scan mode with with a collision energy of 20 eV.

The targeted mass transitions for pyrazines and IS were used for the determination of optimal collision energy in the energy voltages' range of 5, 10, 13, 15, 18, 20, 23, 25, 28, 30, 33, 35, 40, 45, and 50 eV. Dwell times were set to ensure about 15 cycles over a peak.

2.7. Validation of HS-SPME-GC-MS/MS method

The specificity was investigated by comparing the chromatograms for raw and baked JZ with indications of no endogenous interfering compounds under the optimum MRM mode.

The linearity was calculated by the linear regression function and coefficient of determination of the standard curve of pyrazines (0.001, 0.01, 0.1, 1, 10 μg/mL) calibrated by IS.

The sensitivity was determined by LOD, a certain concentration of each pyrazine with signal-to-noise (S/N) over 3. The LOQ (S/N over 10) was determined experimentally to be the lowest spiked concentration that most consistently gave an accuracy of 100 (±20)%.

The accuracy and precision were calculated by the spiking experiments with standard pyrazines (0.05, 0.5, 5 μg/mL) added to JZ and baked JZ within one day and four days, respectively.

2.8. Data analysis

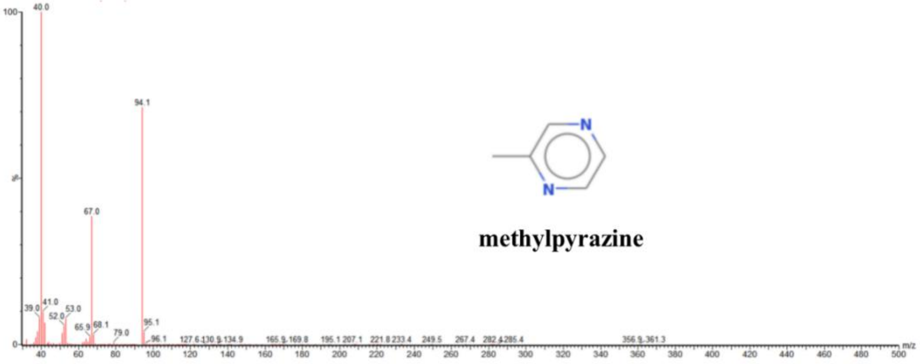
SPSS 21.0 Statistics (SPSS Inc.,Chicago, USA) was used for the analysis of SD (standard deviation). The differences at $p < 0.05$ were considered significant. EXCEL 2013 was used for graphing figures.

3. Results and discussion

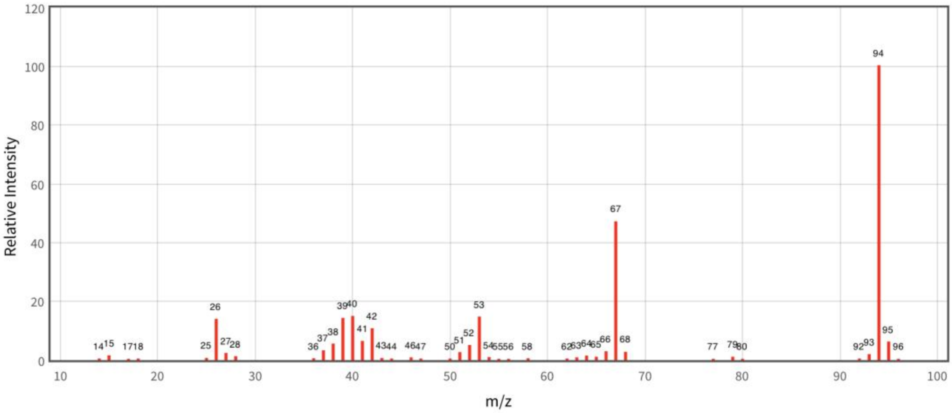
3.1. Optimization of MRM conditions

The mass spectra of pyrazines and IS are shown in Figure 7-1. The precursor ion of methylpyrazine, 2,6-dimethylpyrazine, trimethyl pyrazine, tetramethyl pyrazine, and IS was *m/z* 94.1, *m/z* 108.0, *m/z* 122.1, *m/z* 136, and *m/z* 96.00, respectively.

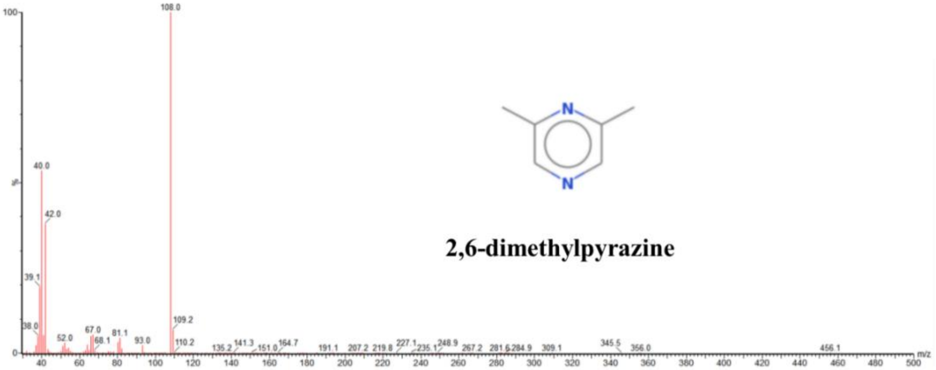
The daughter ions of methylpyrazine, 2,6-dimethylpyrazine, trimethyl pyrazine, tetramethyl pyrazine and IS are reported in Figure 7-2. The quantitative and qualitative transitions were assigned by the mass fragmentation with the two largest peak areas. For instance, the quantitative and qualitative transitions for methylpyrazine were *m/z* 94.1 → 67.0 and m/z 94.1 → 68.1, respectively. The optimization results of collision energy for the four alkyl pyrazines are shown in Table 7-2.The peak area of the quantifier transition (*m/z* 94.1 → 67.0) and qualifier transition (94.1 → 68.1) for methylpyrazine was the highest when the collision energy was 10 eV and 8 eV, respectively. In a parallel manner, the ion pairs of qualitative and quantitative analysis for the four alkyl pyrazines were assigned. The optimization of MS/MS parameters in MRM mode is shown in Table 7-3.



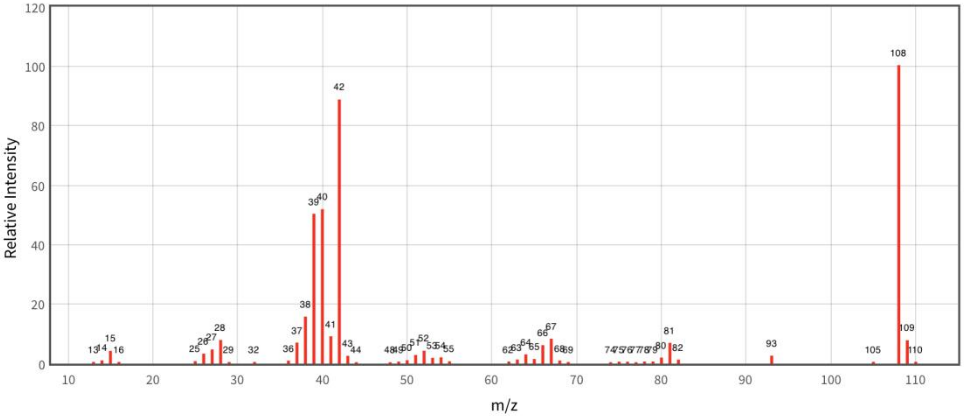
(a1)Mass spectra for methylpyrazine in full scan mode



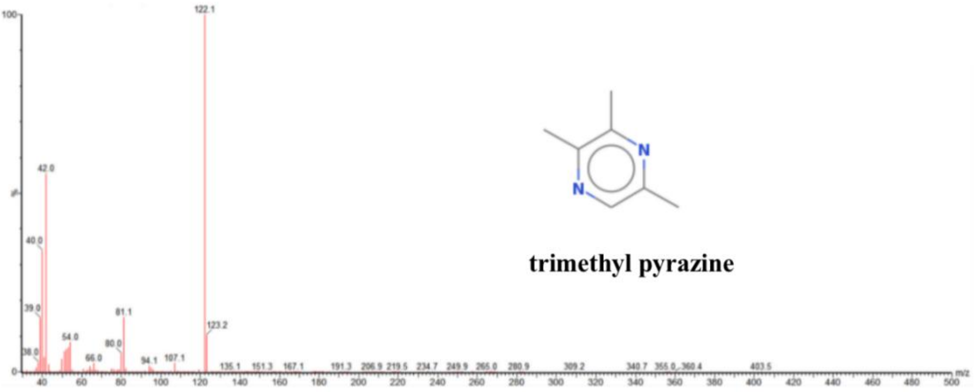
(a2) Mass spectra for methyl pyrazine referred from NIST database



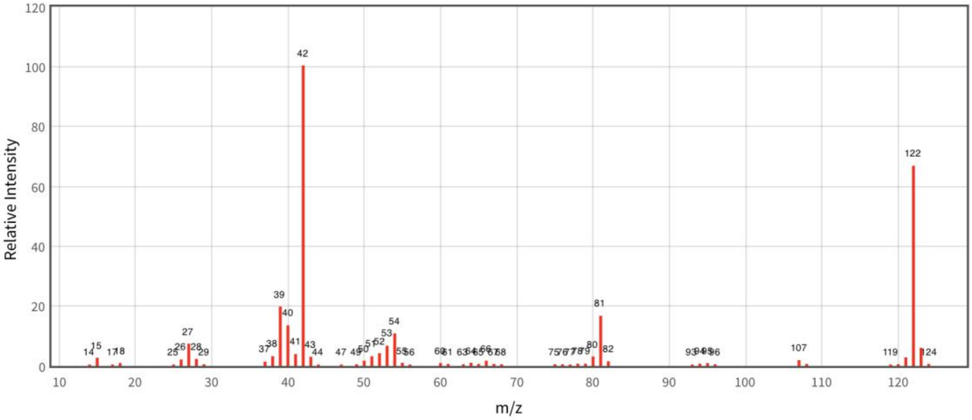
(b1) Mass spectra for 2,6-dimethylpyrazine in full scan mode



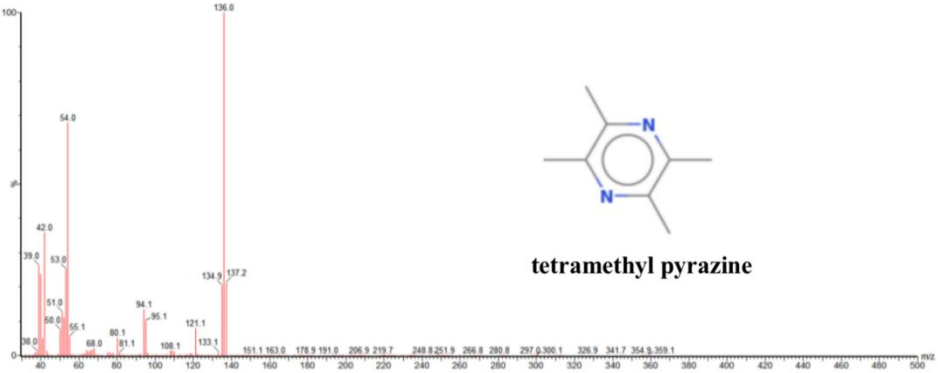
(b2) Mass spectra for 2,6-dimethylpyrazine referred from NIST database



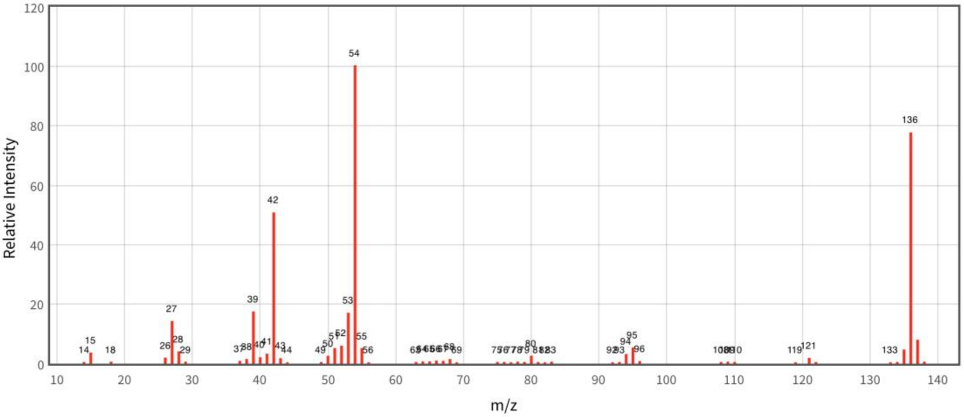
(c1) Mass spectra for trimethyl pyrazine in full scan mode



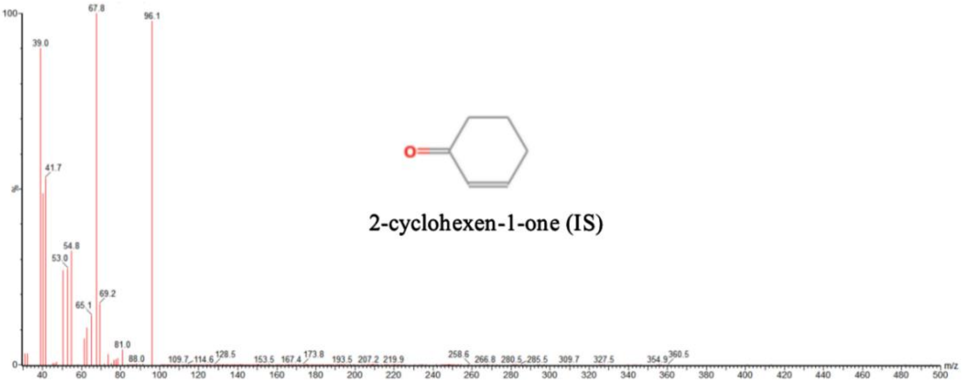
(c2) Mass spectra for trimethyl pyrazine referred from NIST database



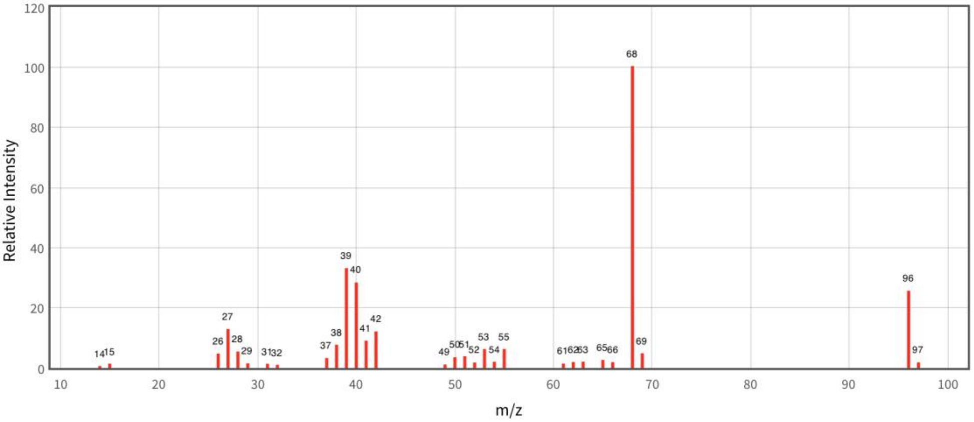
(d 1) Mass spectra for tetramethyl pyrazine in full scan mode



(d 2) Mass spectra for tetramethyl pyrazine referred from NIST database

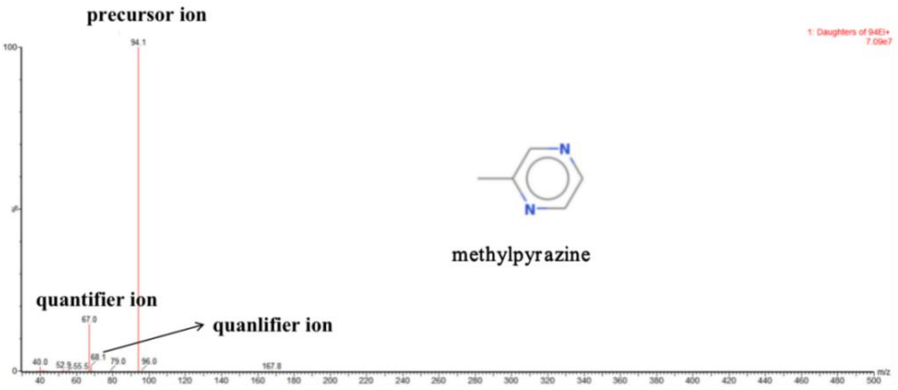


(e1) Mass spectra for IS in full scan mode

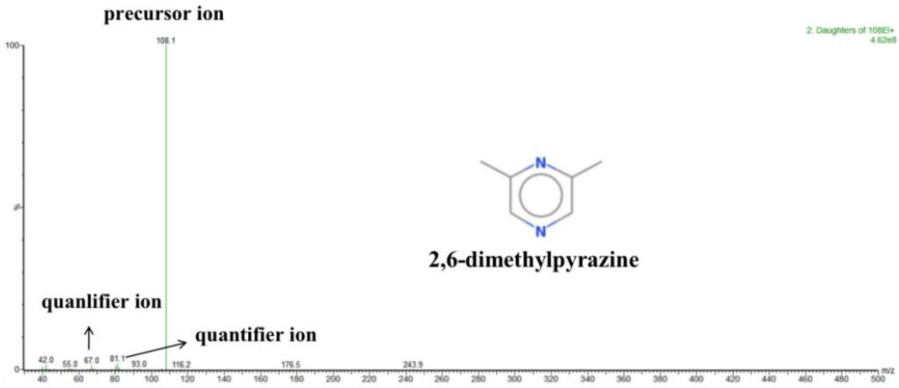


(e2) Mass spectra for IS referred from NIST database

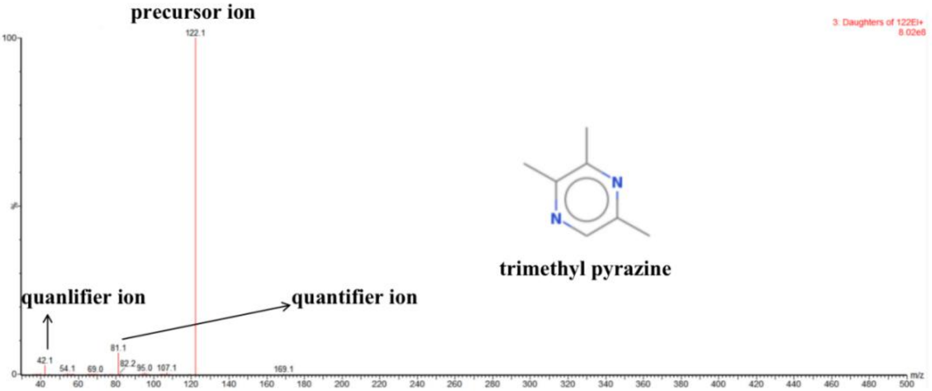
Figure 7-1 (a1) Electron ionization mass spectra for methylpyrazine; (b1) 2,6-dimethylpyrazine; (c1) trimethyl pyrazine; (d1) tetramethyl pyrazine; (e1) IS (internal standard, 2-cyclohexen-1-one). (a2) Mass spectra for methylpyrazine referred from NIST database; (b2) 2,6-dimethylpyrazine; (c2) trimethyl pyrazine; (d2) tetramethyl pyrazine; (e2) IS (internal standard, 2-cyclohexen-1-one).



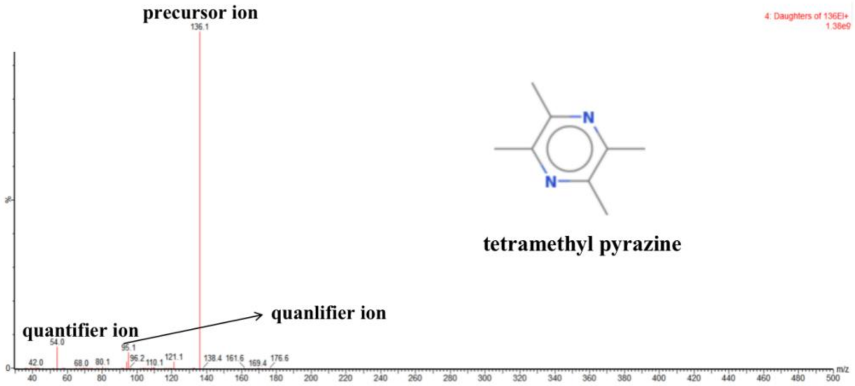
(a) Daughter ion scan of methylpyrazine.



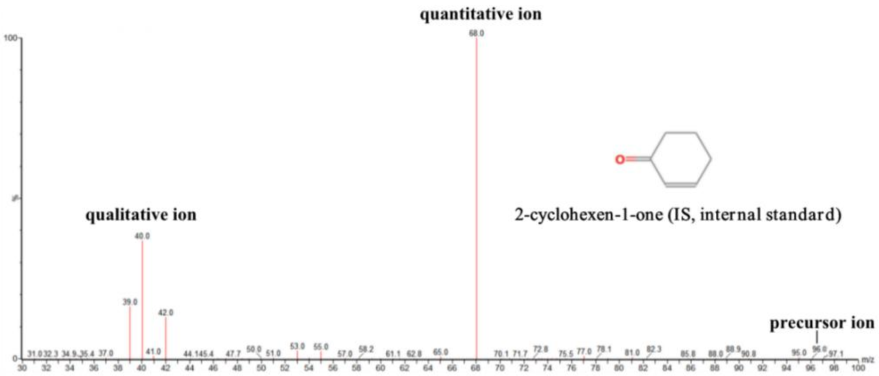
(b) Daughter ion scan of 2,6-dimethylpyrazine.



(c) Daughter ion scan of trimethylpyrazine.



(d) Daughter ion scan of tetramethyl pyrazine.



(e) Daughter ion scan of internal standard (IS, 2-cyclohexen-1-one)

Figure 7-2 HS-SPME-GC-MS/MS chromatograms (a) methylpyrazine in a daughter ion scan mode with precursor ion of *m/z* 94.1; (b) 2,6-dimethylpyrazine in a daughter ion scan mode with precursor ion of *m/z* 108; (c) trimethyl pyrazine in a daughter ion scan mode with precursor ion of *m/z* 122.1; (d) tetramethyl pyrazine in a daughter ion scan mode with precursor ion of *m/z* 136 (e) 2-cyclohexen-1-one (IS, internal standard) in a daughter ion scan mode with precursor ion of *m/z* 96.

Table 7-2 The optimization of collision energy for alkyl pyrazines.

compound	transition	Peak Area (arbitrary unit)															
		5 eV	8 eV	10 eV	13 eV	15 eV	18 eV	20 eV	23 eV	25 eV	28 eV	30eV	33eV	35eV	40eV	45eV	50eV
methylpyrazine	94.1 → 67.0	1530000±125231	5760000±345233	5980000±434500	4460000±34569	2250000±33455	1190000±7600	449000±32345	228000±15443	87600±4567	27700±2065	14010±1040	12500±657	8020±764	3820±32	2900±213	1627±152
	94.1 → 68.1	188000±12134	495000±23460	365000±24300	231000±8934	103000±7535	52600±3215	19600±1234	10900±467	73930±3456	31110±2164	12260±898	10100±347	4330±324	2070±198	1830±132	683±56
2,6-dimethyl pyrazine	108.1 →67.0	885±54	4170±326	3440±213	2800±112	1500±102	1060±58	642±35	351±11	136±11	104±10	98±11	85±9	76.2±54	59.1±5	48.8±5	27.9±4
	108.1 → 81.1	1080±74	3320±245	4880±323	2650±106	1280±112	938±67	512±21	319±34	172±14	103±12	88±10	76±6	55.8±32	37.4±3	32.4±5	24.1±3
trimethyl pyrazine	122.1 → 42.1	855±54	5220±453	13900±874	27400±1034	23400±124	15300±856	9160±456	8560±756	5470±234	3570±312	2940±230	1740±114	871±65	610±50	386±22	213±15
	122.1 → 81.1	1310±76	4360±432	7910±611	12300±765	4980±265	2010±112	765±56	409±21	354±22	264±21	167.4±112	99±7	65.5±6	54.5±5	46.4±4	36.8±3

tetramethyl pyrazine	136.1→54.0	720±56	8460±765	17600±432	95800±3452	40600±3215	21000±1045	13500±980	6192±324	4170±331	2800±15	1520±132	808±7	349±32	215±15	188±17	177±13
	136.1→95.1	628±43	2300±206	3530±112	10400±534	7435±602	4210±325	2600±124	1667±121	1173±109	526±27	393±32	270±23	128±11	80±7	68±5	29±4

Table 7-3 The qualitative and quantitative information of individual alkyl pyrazines.

Compounds	Retention time (min)	Qualitative ion pair (*m/z*)	Quantitative ion pair (*m/z*)	Qualitative collision energy (eV)	Quantitative collision energy (eV)
methylpyrazine	6.51	94.1 → 68.1	94.1 → 67.0	8	10
2,6-dimethylpyrazine	9.35	108.0 →67.0	108.0 →81.1	8	10
trimethyl pyrazine	12.11	122.1 →42.1	122.1 →81.1	13	13
tetramethyl pyrazine	14.79	136.1 → 95.1	136.1 →54.0	13	13
2-cyclohexen-1-one	9.77	96.00 → 40.00	96.00 → 68.00	20	20

‘*’represented the relative abundance ratio of qualitative ions to quantitative ions (n=3).

3.2. Optimization of HS-SPME conditions

The fiber coatings, extraction time and temperature were optimized to maximize the extraction yield of pyrazines at least below the odor threshold (in water) of methylpyrazine (60 µg/g), 2,6-methyl pyrazine (0.2 µg/g), trimethyl pyrazine (0.4 µg/g) and tetramethyl pyrazine (0.01 µg/g) (Javier & Maria, 2019). A volume of 10 µL mix standards (10 µg/mL) and 10 µL 2-cyclohexen-1-one (IS, 10 µg/mL) added in the pyrazine free red jujube matrix was used for the test. The HS-SPME condition of fiber coatings was optimized at an extraction temperature of 50 °C for 30 min after a 40 min, 300 rpm equilibration period. The column of DB-5 was used in this study, which was in accordance with the study that focused on the influence of precursors on the formation of pyrazines conducted by Scalone et al. (Scalone, Cucu , De Kimpe, & De Meulenaer, 2015). The extraction efficiency of each fiber, namely DVB/CAR/PDMS, CAR/PDMS, PDMS/DVB, PDMS and PA were shown in Figure 7-3. The most suitable fiber for the extraction of methylpyrazine, 2,6-dimethyl pyrazine, trimethyl pyrazine, and internal standard was CAR/PDMS as the peak areas were the highest when using this fiber. In general, CAR/PDMS was selected as the fiber coating for simultaneous determination of the pyrazines in this study. Similarly, the extraction efficiency of pyrazines from roasted green tea was superior using CAR/PDMS as extraction fiber (Yang et al., 2020).

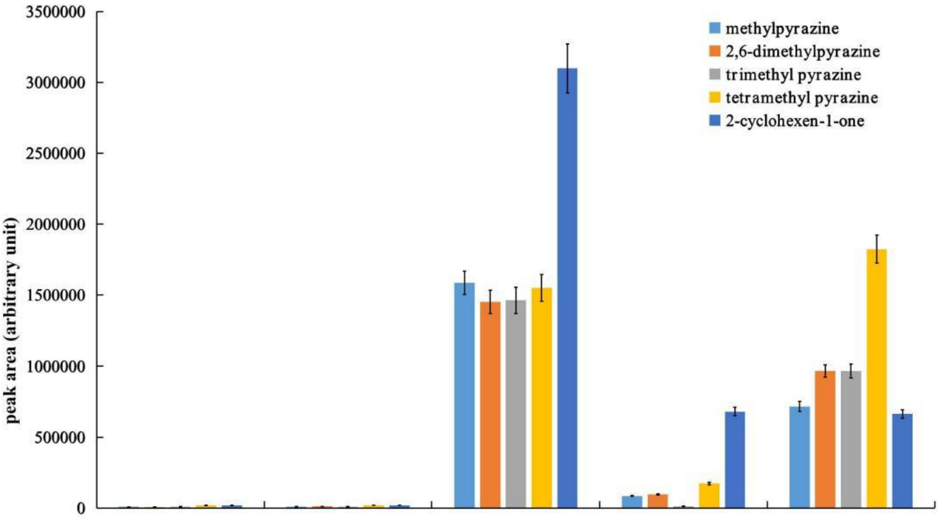


Figure 7- 3 Effects of types of SPME fibers on four alkyl pyrazines (CAR, carboxen; PDMS, polydimethylsiloxane; DVB, divinylbenzene; PA, polyacrylate).

A volume of 10 µL mix standards (10 µg/mL) and 10 µL 2-cyclohexen-1-one (IS, 10 µg/mL) were added in the pyrazine free red jujube matrix for the test. For the step of SPME, the adsorption time and temperature were optimized for the sensitive and fast detection of pyrazines, as well as the the extraction of IS (Figure 7-4). For the following experiments, the conditions CAR/PDMS fiber, equilibration period of 40 min with the agitation speed of 300 rpm, and a weight of 2.00 ± 0.02g raw JZ were used. With the increase of adsorption temperature (30 °C and 50 °C), the area of pyrazines in raw JZ and IS increased gradually. When the adsorption temperature increased from 50 °C and 70 °C, the area of pyrazines decreased. Thus the adsorption efficiency of pyrazines and IS was the highest when the extraction temperature was 50 °C. Under the extraction temperature of 50 °C, it was observed that the area of 2,6-dimethyl pyrazine increased gradually when the extraction time was extended from 5 min to 25 min. However, when it extended to 40 min, the extraction efficiency was not significantly improved. For the extraction of the other pyrazines, the peak area did not show a significant increase when the extraction time increased. On the other hand, for the extraction of IS, the peak area was the highest when the adsorption time was 25 min, although its peak area did not change regularly over time. Finally, the optimal extraction condition was determined using a temperature of 50 °C and a time of 25 min.

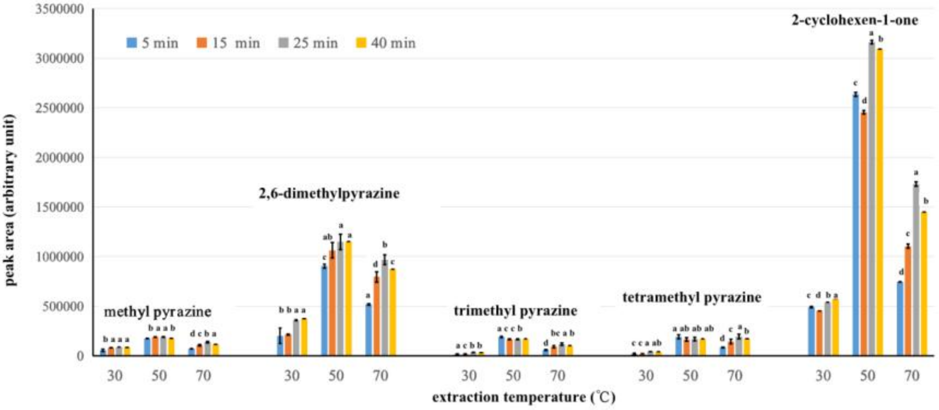


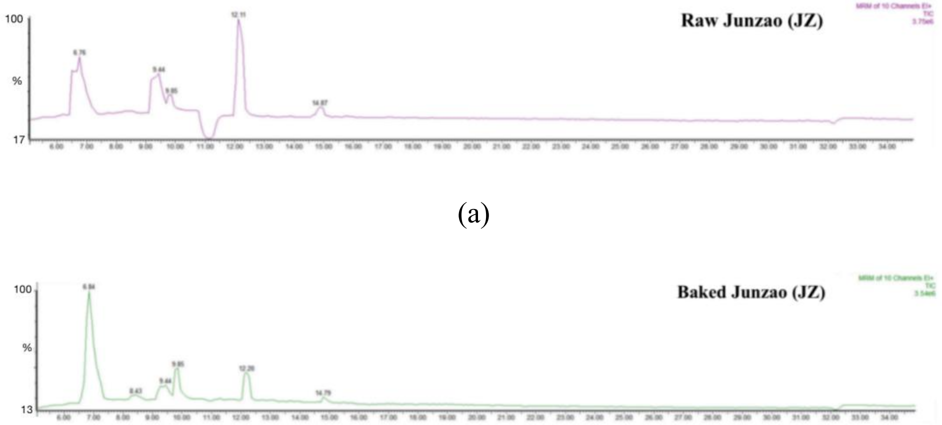
Figure 7-4 Comparison of extraction time (5, 15, 25, 40 min) and temperature (30, 50, 70 °C) for four kinds of pyrazines in raw Junzao and 2-cyclohexen-1-one. Error bars were standard deviations (n = 3). A volume of ten µL 2-cyclohexen-1-one was used as internal standard (IS))($p<0.05$).

3.3. Validation of HS-SPME-GC-MS/MS method

The fiber coatings, extraction time and temperature were optimized for a condition to get the maximum detection rate of pyrazines, which was below the odor threshold (in water) of methylpyrazine (60 µg/g), 2,6-methyl pyrazine (0.2 µg/g), trimethyl pyrazine (0.4 µg/g) and tetramethyl pyrazine (0.01 µg/g) (Javier & Maria, 2019). A volume of 10 µL mix standards (10 µg/mL) and 10 µL 2-cyclohexen-1-one (IS, 10 μg/mL) added in the pyrazine free red jujube matrix was used for the test. The HS-SPME condition of fiber coatings was optimized at an extraction temperature of 50 ℃ for 30 min after a 40 min, 300 rpm.

3.3.1 Specificity

In spite of the ameliorative conditions that affected the analysis of HS-SPME, the effect of food matrix was also crucial for the qualitative and quantitative analysis. Matrix effect, as well as inlet liner and column were the factors affecting the analysis of GC, causing quantitative errors (Saka et al., 2013). The response values of standards in neat solvent may be different from those in a matrix or solution, which may contain coextractives that can cover up the potential reaction points including silanol groups and metal ions in GC, enabling more analytes to be detected (Hajšlová & Zrostlíková, 2003). Thus, the addition of standards in pyrazine-free matrix was chosen for the addressing of matrix effect. The chromatogram of raw and baked JZ were listed for the considering of specificity (Figure 7-5).



(a)

(b)

Figure 7-5 The chromatogram of (a) raw and (b) baked Junzao (n=3)

3.3.2 Linearity, LOD, LOQ

The evaluation of calibration curves, linearity range, LOD, and LOQ was performed by analyzing standard curves in the ranges of 0.001-10 μg/mL (methylpyrazine, trimethyl pyrazine, and tetramethyl pyrazine) and 0.01-10 μg/mL for 2,6-dimethyl pyrazine, using pyrazine-free matrix as calibration medium (Table 7-4). The LOD (0.5-10 pg/g) of the four alkyl pyrazines were far below their odor thresholds (0.01-60 μg/g).

Table 7-4 Calibration curves, linear ranges, correlation coefficients, limits of detection (LOD), and limits of quantification (LOQ) for alkyl pyrazines.

compounds	calibration curves	linear ranges (μg/mL)	R^2	LOD (pg/g)	concentration of the calculated S/N (ng/mL)	S/N (quantifier transition)	S/N (qualifier transition)	LOQ (pg/g)	concentration of the calculated S/N (ng/mL)	S/N (quantifier transition)	S/N (qualifier transition)
methylpyrazine	$y = 1.6026x - 0.1185$	0.001-10	0.9988	0.5	0.1	24.42	5.13	1.5	0.3	62.67	13.16
2,6-dimethyl pyrazine	$y = 0.3305x + 0.7334$	0.01-10	0.9947	10	2	28.38	6.05	30	6	56.29	12.00
trimethyl pyrazine	$y = 1.4712x + 0.3032$	0.001-10	0.9952	0.5	0.1	23.79	8.09	1.5	0.3	34.21	11.63
tetramethyl pyrazine	$y = 4.0961x - 0.3613$	0.001-10	0.9983	0.5	0.1	19.85	5.26	1.5	0.3	90.33	23.90

3.3.3 Precision and accuracy

The intraday and interday precision and accuracy of alkyl pyrazines is shown in Table 7-5. A volume of 10 μL mixed pyrazine standard (0.05, 0.5 and 5 μg/mL) was added in raw and baked JZ to evaluate precision by RSD (%), and to evaluate accuracy by recovery. The RSD of the intraday and interday repeated analysis were ≤ 10% and ≤ 12%. The bias ranged from 2.7 % to 13.2 %. In general, the results indicate a reasonable precision and accuracy of the developed method for the determination of alkyl pyrazines in raw and baked red jujube.

Table 7-5 Precision and accuracy of alkyl pyrazines obtained by HS-SPME-GC-MS/MS

Sample	Concentration (μg/mL)	Intraday (n=7)*		Interday (n=12)**	
		Bias (%)	RSD (%)	Bias (%)	RSD (%)
Raw Junzao (JZ)	0.05	7.0	8.4	4.6	11.2
	0.5	10.6	5.1	2.7	8.5
	5	13.2	4.6	7.7	5.5
Baked Junzao (JZ)	0.05	3.8	3.8	5.4	7.0
	0.5	6.4	6.5	4.5	4.2
	5	2.8	9.4	5.7	7.1

‘*’The intraday experiment was conducted with standard solutions added in raw and baked Junzao by seven times repeated analysis at the same day.

‘**’The interday experiment was conducted with standard solutions added in raw and baked Junzao by three times repeated analysis in four different days.

3.4. Alkyl pyrazines in raw and baked red jujube

The determination of alkyl pyrazines in five cultivars of red jujubes were carried out for the evaluation of the method on accounts of reliability and suitability. The information of the red jujube samples was shown in Table 7-1. The data showed that pyrazines existed in different concentrations of raw and baked red jujube samples (Table 7-6). For instance, methylpyrazine was tested in all red jujube samples with the content ranging from 42.25 to 916 μg/g. 2,6-dimethylpyrazine presented a relatively narrow concentration range varied from 43.83 to 143.12 μg/g, whereas trimethylpyrazine displayed an extremely high broad range from 58.01 to 1784.37 μg/g. For tetramethyl pyrazine, it presented a range of 8.37 to 234.77 μg/g in raw and baked red jujube. Therefore, the intake of raw red jujube in consideration of these four alkyl pyrazines should be no more than 1028 g/kg body weight. However, tetramethyl pyrazine could not be detected in raw MSDZ and baked LWCZ and JZ owing to the extremely low concentrations (< LOQ).

Table 7-6 Alkyl pyrazine composition in raw and baked red jujube (n=3)

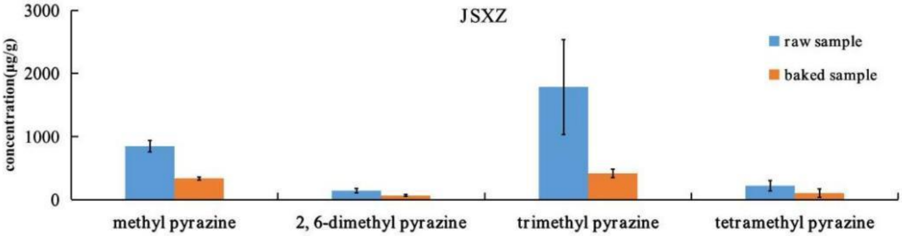
samples		Content (μg/g)			
		methylpyrazine	2,6-dimethyl pyrazine	trimethyl pyrazine	tetramethyl pyrazine
raw sample	JSXZ	849.28±93.28ab	143.12±14.75a	1784.37±151.34a	222.2±11.07ab
	MSDZ	42.25±1.73e	49.59±2.47e	58.01±6.58e	< LOD
	HZ	916.00±54.3a	121.73±7.71ab	943.66±96.46b	234.77±18.04a
	LWCZ	357.98±59.44c	86.92±4.78c	327.3±11.97c	31.92±0.55d
	JZ	140.7±4.34d	67.00±2.34d	268.98±14.67d	62.26±1.61c
baked sample	JSXZ	335.98±22.85ab	67.06±5.95ab	417.28±35.67a	103.49±9.14a
	MSDZ	86.73±1.18d	43.83±6.87cd	35.35±2.84e	8.37±0.17c
	HZ	349.99±32.17a	71.26±6.4a	294.3±22.61b	59.14±5.41b
	LWCZ	143.58±9.9c	57.32±4.73b	96.8±8.52c	< LOD
	JZ	51.55±4.18e	47.53±4.62c	46.05±6.56d	< LOD

Note: JSXZ, Jinsixiaozao; HZ, Huizao; LWCZ, Lingwuchangzao; JZ, Junzao; MSDZ, Mingshandazao.

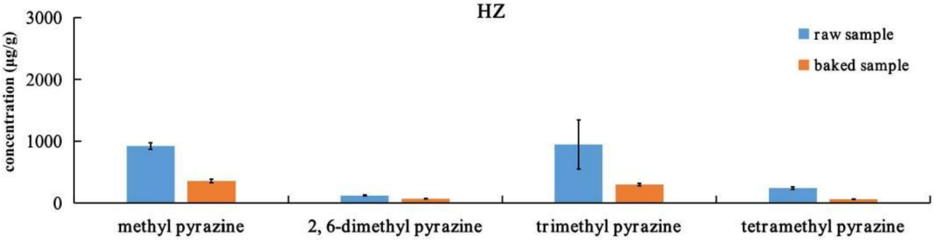
The significance of alkyl pyrazines in different cultivars of raw and baked red jujubes was expressed as $p < 0.05$.

3.5. Effect of baking on the composition of alkyl pyrazines

The changes of alkyl pyrazines in raw and baked red jujube were depicted in Figure 7-6. Generally, trimethylpyrazine was the highest in raw red jujube except for LWCZ, accounting for 38.71% ~ 59.50% of the total pyrazine. However, in addition of baked JSXZ, the content of methylpyrazine in baked red jujube was the highest, accounting for 35.52% ~ 49.76% of total pyrazine content. The same phenomenon was found in coffee. Commercial coffee, which also undergoes the roasting process (over 200 ° C), contained the highest proportion of 2-methylpyrazine of all alkylpyrazines (Pickard, Becker, Merz, & Richling, 2013; Kwon, Ahn, & Lee, 2021). In detail, raw JSXZ contained the highest amount of 2,6-dimethyl pyrazine (943.66 μg/g) and trimethyl pyrazine (1784.37 μg/g). Raw HZ appeared to have more methylpyrazine (916 μg/g) and tetramethyl pyrazine (234.77 μg/g). For the pyrazines in baked samples, methylpyrazine (349.99 μg/g) and 2,6-dimethyl pyrazine (71.26 μg/g) were found in higher concentrations in baked HZ. Trimethyl pyrazine (417.28 μg/g) and tetramethyl pyrazine (103.49 μg/g) were more abundant in baked JSXZ than in other varieties. Thus, the calculated daily intake of baked red jujube in terms of the four alkyl pyrazines was 5143 g/kg body weight. On the other hand, the content of methylpyrazine, 2,6-dimethyl pyrazine, and trimethyl pyrazine in JZ, HZ, JSXZ and LWCZ decreased after the baking process, except for the increase of methylpyrazine and tetramethyl pyrazine in MSDZ.



(a)



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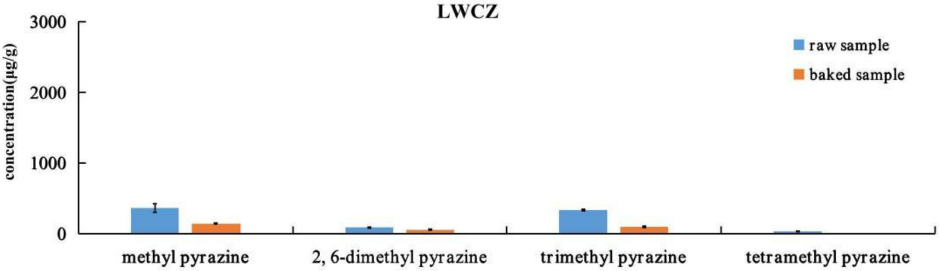

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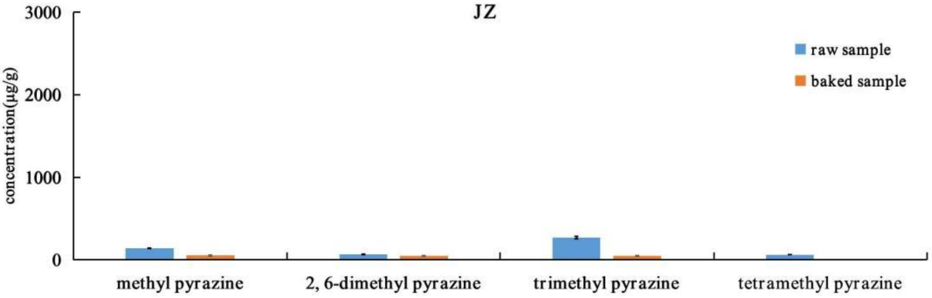

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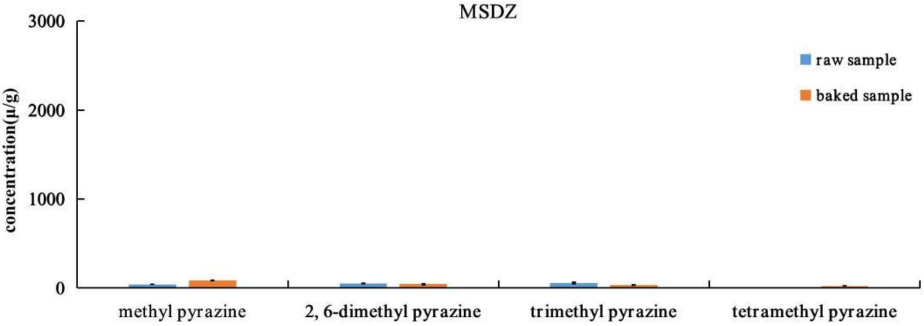

(e)

Figure 7-6 The changes of methylpyrazine, 2,6-dimethyl pyrazine, trimethyl pyrazine, and tetramethyl pyrazine in five cultivars of raw and baked red jujube (a) JSX, Jinsixiaozao; (b) HZ, Huizao; (c) LWCZ, Lingwuchangzao; (d) JZ, Junzao; (e) MSDZ, Mingshandazao)

Pyrazines are widely distributed in natural organisms such as foodstuffs, plants, water, animals, and microorganisms (Rajini, Aparna, Sasikala, & Ramana, 2011). For example, methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, trimethylpyrazine, 2-ethylpyrazine, 2-methoxy-3,5-dimethylpyrazine, and 2-ethyl-3,6-dimethylpyrazine were previously found in red jujubes (cv. 'Jinsixiaozao', 'Youzao', and 'Yuzao'). Moreover, 2,5-dimethylpyrazine and 2,6 -dimethylpyrazine were identified as two of the key odor active compounds (Zhu & Xiao, 2018). Generally, pyrazines in foodstuffs are formed in an anthropogenic way, especially in thermal processing (above 100 ° C) (Shibamoto & Bernhard, 1977). A variety of pyrazines rendered between α-dicarbonyl and amino acids through condensation function (Shibamoto & Bernhard, 1977). For example, as is shown in Figure 7-7, dihydropyrazine (7) could be condensed from two α-aminocarbonyl compounds (4 or 6) (Kocadagli, Methven, Kant, & Parker, 2021). And then the deprotonation and aldolization of dihydropyrazine (7) may yield alkylpyrazine (11) and the amino-acid-specific pyrazine (12) (Amrani-Hemaimi, Cerny, & Fay, 1995; Scalone, Cucu, De Kimpe, & De Meulenaer, 2015). At last, acylated pyrazines (13) and the substituted vinylpyrazine (14) might form in the following reactions (Adams et al., 2009; Kamal, Neubert, & Levine, 1962).

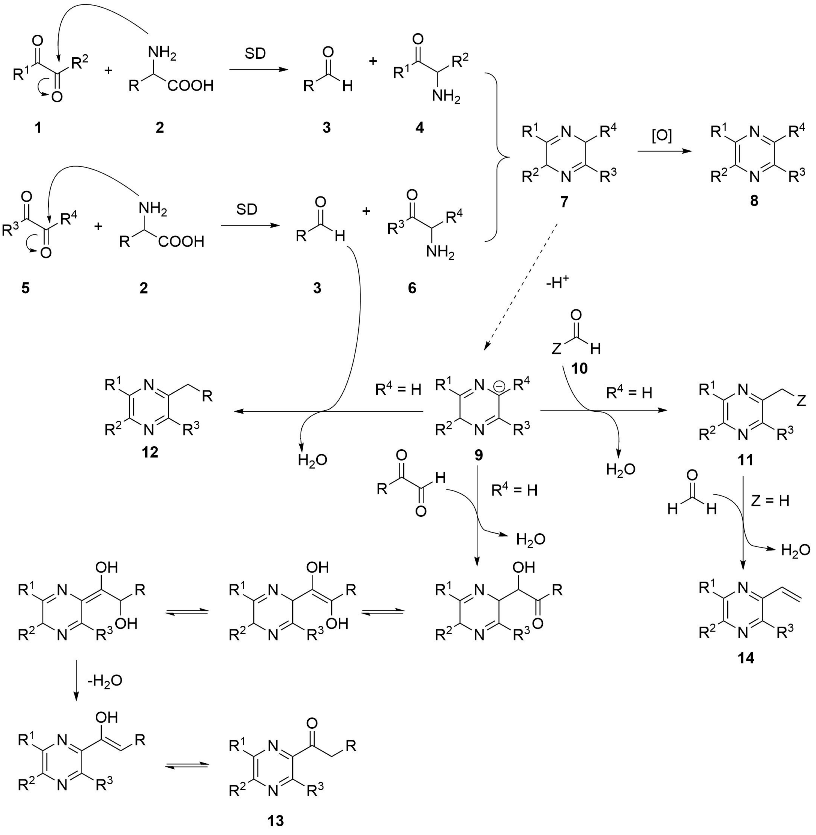

Figure 7-7 The proposed mechanism for pyrazines formation

The degradation of pyrazines is mainly divided into the following processes: photocatalytic and biological degradation, oxidative degradation, reductive degradation, and channelization of pyrazines (VRajini, Aparna, Sasikala, & Ramana, 2011). For example, the high temperature (180 °C) in the baking process might be a stimuli for the dealkylation, resulting in the relative lower levels of methylpyrazine, 2,6-dimethyl pyrazine and trimethyl pyrazine (Müller & Rappert, 2010). Furthermore, the polar (heteroatoms) and non-polar (aromatic moieties) fragments of pyrazines are the most frequent groups that interacts. For example, the intermolecular interaction of pyrazines happens in the bonding of hydrogen to nitrogen atoms. Besides, there are several ways for the interaction of pyrazines and other compounds. For example, the hydrogen bond of pyrazines can interact with nitrogen atoms of other compounds. The nitrogen atoms of pyrazines can also interact with metals in proteins (Juhás & Zitko, 2020). Therefore, the reduction of some pyrazines after the baking of red jujube might also be related to the reaction of pyrazines with pyrazines and proteins. For the increase of tetramethyl pyrazine in MSDZ, it might be a result of the accumulation of acetoin, which was the precursor of tetramethyl pyrazine (Hao, Wu, &Xu, 2013).

4. Conclusion

A method for tracking of the distribution of the four alkyl pyrazines in red jujube during the baking process was established by HS-SPME-GC-MS/MS with specificity, precision, accuracy, sensitivity, and linearity with a wide range of concentration. Trimethylpyrazine and methylpyrazine were the most abundant alkyl pyrazines in raw and baked red jujube, respectively. The contents of methylpyrazine, 2,6-dimethyl pyrazine, and trimethyl pyrazine were reduced during the baking of red jujube (HZ, JZ, JSXZ, and LWCZ). The thermal processing of baking (180 °C, 4min) activated the formation of tetramethyl pyrazine in MSDZ. Alkylpyrazines presented a class of aroma characteristics such as nutty, roasted, sweet and musty notes, as well as biological activities such as antioxidant and anti-inflammatory, which were important for food quality and consumer acceptance. The method established in this paper can be applied to the dynamic monitoring of pyrazine in food matrix containing red jujube.

8

Chapter VIII. General discussion, conclusion and perspective

The untargeted and targeted aroma profile analysis undertaken in this thesis demonstrated the aroma profile of winter jujube from different regions and red jujube from Xinjiang Province, China, the baking induced changes on the key odor active compounds in red jujube, together with the development of the detection methods for several specific heterocyclic compounds (pyran and pyrazines). The research was carried out through the techniques of GC-IMS, E-nose, HS-SPME GC-MS, GC-O-MS, sensory evaluation, and HS-SPME-GC-MS/MS. Benzyl alcohol, octanoic acid, 2-hexenal, linalool, 2-nonenal, and ethyl decanoate were the most common compounds presented in the winter jujubes from different regions of China. The presence of acetoin, E-2-hexanol, hexanal, acetic acid, and ethyl acetate played an important role in the classification of the red jujube cultivars from Xinjiang Province, China. The baking process triggered the formation of aromatic hydrocarbon molecules in red jujube including 1H-pyrrole-2-carboxaldehyde, 2-hexylthiophene, 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, 5-methyl-2-furancarboxaldehyde, and benzaldehyde.

1. General discussion

1.1. The aroma characteristics of winter jujube from eight different regions of China

Winter jujube, as the most prominent elite variety of fresh jujube (*Zizyphus jujuba* Mill.), is deeply favored by people for its unique flavor, good taste, and nutritional value including vitamin C, amino acids, cyclic AMP, carbohydrate, and minerals (Hui et al., 2015; Gu et al., 2005). Winter jujube is a high-yielding variety, ranking as one of China's greatest economic value fruit in terms of production, trade, and processing (Gu et al., 2005; Li, 2010). And it is widely cultivated in many provinces of China (Zhou et al., 2019). Among the factors like genotype (cultivar), geographical conditions, postharvest storage conditions, processing, and the degree of maturity at harvest that affect the physicochemical quality of fruits, the effect of geographical conditions on winter jujube aroma has been firstly studied in this project (Wang et al., 2013). The VOCs of benzyl alcohol, octanoic acid, 2-hexenal, linalool, 2-nonenal, and ethyl decanoate have been identified as the common VOCs of winter jujubes from eight regions (Hebei Province, Huanghua City; Shandong Province, Zhanhua City; Shandong Province, Yantai City; Shanxi Province, Yuncheng City; Shanxi Province, Linyi City; Shannxi Province, Dali City; Xinjiang Uygur Autonomous Region, Kuerle City; Xinjiang Uygur Autonomous Region, Akesu) of China with the application of GC-IMS, E-nose, and GC-MS. The aroma properties of fruity, grass, or green presented by these VOCs were consistent with the sensory characteristics studied in winter jujube from previous studies (Pu et al., 2011). Meanwhile, the presence of 1-penten-3-ol, ethyl hexanoate, methyl laurate, and 2-formyltoluene were crucial for the geographical differentiation of the winter jujubes, showing SXYC and XJAKS were individually apart from the other winter jujubes. The green and fruity aroma profile of SXYC could be correlated with the presence of 1-penten-3-ol, ethyl hexanoate, methyl laurate, and 2-formyltoluene. Furthermore, the presence of acetone and 2-methoxyphenol was identified as the potential markers of XJAKS. The study on the effect of geographical distribution on winter jujube aroma profile provides volatile indicators of fresh jujube's aroma quality. It also reflects the effects of geographical conditions on the metabolism and accumulation of volatile chemicals, which could be useful for the cultivation points' choices. For example, the compounds of acetone and 2-methoxyphenol were rather important for the aroma of winter jujubes cultivated in XJAKS. The general information of winter jujube aroma reflects the points that should be protected in winter jujube processing. For example, the compounds of 1-penten-3-ol, ethyl hexanoate, methyl laurate, and 2-formyltoluene should be kept for the green and fruity aroma of the final product. Besides, the identification of these specific compounds would be useful for the imitation of distinctive aroma products derived from winter jujube.

1.2. The volatile profile of red jujubes from Xinjiang Province, China

Red jujube is the dried form of jujube with various health benefits and delicious taste. It is native to China and has been cultivated extensively in tropical and subtropical regions (Rashwan et al., 2020). There are more than 700 cultivars of red jujube distributed in the six major production regions of China, namely Xinjiang Province, Hebei Province, Henan Province, Shandong Province, Shanxi Province, and Shannxi Province (Liu & Zhao, 2009). Among these areas, Xinjiang Province is the biggest one, with two million hectares of cultivation that produce approximately 37.2% of the global red jujube market (Yu et al., 2020). This study was to investigate the particular aroma profile of the red jujube cultivated in Xinjiang Province and correlate the sensory characteristics with a chemical composition including VOCs, fatty acids, amino acids, organic acids, and sugars. GC-IMS, multivariate data analysis, and descriptive sensory analysis, and correlation analysis were applied in the project. The results showed the aroma profile of six red jujube cultivars (QYX, JC, HTDZ, YZ, HZ, and JZ) collected from Xinjiang Province was composed by sixty-four compounds. Sixteen compounds including hexanoic acid, isobutyric acid, 3-methylbutanoic acid, n-decanoic acid, heptanoic acid, 1-octen-3-ol, hexanal, benzaldehyde, E-2-heptenal, n-nonanal, 6-methyl-5-hepten-2-one, ethyl hexanoate, ethyl benzoate, methyl benzoate, 2-pentyl furan, and o-cymene) had been identified in red jujube in the previous study carried out by Chen et al. (2018). The VOCs of acetoin, E-2-hexanol, hexanal, acetic acid, and ethyl acetate were crucial in the discrimination of the different cultivars of red jujube from Xinjiang Province. In terms of sensory notes, HZ showed relatively higher scores in fruity (6.4) and floral (1.5) attributes. JZ achieved a higher value on sweet (7.8), sour (3.2) and jujube ID (8.0) attributes. HTDZ got higher scores in bitter (0.8) and astringent (1.0) attributes. Nevertheless, JC, QYX, and YZ showed higher values in jujube ID (8.0), sweet (7.2) and sour (3.0), respectively. The composition of VOCs, amino acids, fatty acids, and organic acids could explain the variation in sensory notes in part. The correlation analysis highlighted the possibility of the contribution of phenylacetaldehyde and isobutanoic acid to the jujube ID, as referred from the findings that jujube ID was mainly correlated with the existence of amino acids (THR, GLU, GLY, ALA, VAL, LEU, TYR, PHE, LYS, HIS, ARG) and free amino acids may transaminate or dehydrogenate into aldehydes or acids (phenylacetaldehyde and isobutanoic acid) (Schwab et al., 2008; Jelen, 2011). The results reflect red jujubes of a given cultivation area share specific common volatiles that can serve as a fingerprint for different species cultivated in Xinjiang Province, China. The sensory quality evaluation provides information on raw red jujube and consequently of the processed final products, eg. JZ with higher sweety and jujube ID intensities could be consumed directly, or HTDZ with more assertive bitterness and astringency could be processed into powders as addition of jam or candies.

1.3. The baking induced changes in key odorants of red jujube

Jujube fruits are eaten fresh, dried, or processed (Krška & Mishra, 2008). Fresh jujube fruit is smooth and bright, with sweet and crisp flesh (Liu et al., 2020). Dried jujube is the main form of jujube product, and it appears wrinkled and it is described as sweet, fruity, sour, and a bit bitter (Wojdyło et al., 2016). The various processing methods were firstly reported by Shin et al. in 1992 (Shin et al., 1992). The processed jujube (cloying with honey, preservation in vinegar, and conservation in compote) was evaluated as sweet and sour (Krška & Mishra, 2008). During the production of some traditional foods such as jujube cakes and jujube tea, the baking process imparts an attractive, strong, and pleasant aroma to these jujube-based products. The baking process has been described to contribute to the roasted and caramel-like aroma of beans and hazelnut (Rainey, 1986; Bi et al., 2021). However, the baking effect on the aroma profile of jujube has not been evaluated and elucidated until now. The cultivar of Junzao from the famous and most extensive producing area, Xinjiang Province, China, was selected as the object of this study, for its superior quality and since it is widely consumed. The baking condition used was 180 °C, 4 mins, based on the color acceptance and aroma intensity outcomes. The results showed that six compounds contributed to the overall aroma of raw red jujube, namely heptanal, benzeneacetaldehyde, β-damascenone, hexanoic acid, 5-butyltetrahydro-2-furanone, and pentanoic acid, contributed much to the overall aroma of raw red jujube, while 5-methyl-2-furancarboxaldehyde, β-damascenone, benzaldehyde, limonene, hexanoic acid, 5-butyltetrahydro-2-furanone, and DDMP were the key odorants for baked red jujube. The baking process enhanced the intensity of sweet notes and gave rise to caramel and roast-related notes, which might be caused by the presence of 5-methyl-2-furancarboxaldehyde and benzaldehyde.

1.4. The detection of DDMP (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one) in baked red jujubes by HS-SPME-GC-MS/MS

Thermal treatments are extensively used in the food industry and may cause the degradation of food quality (Ling et al., 2015). There may be chemical reactions in the thermal processing that lead to aroma or color changes. In the baking process of red jujube, DDMP with a bitter odor and potential toxicity was studied to be formed after the 2,3-enolization and Amadori rearrangement products oxidation (Li et al., 2019a). Thus it is necessary to develop a method for the qualitative and quantitative characterization of DDMP in thermal processed red jujube. The method developed in this project was based on HS-SPME-GC-MS/MS for its advantages of higher sensitivity and selectivity with minimal sample preparation (Yost & Fetterolf, 1983). The developed method for the detection of DDMP in the baking process was applied successfully. The standard curve was linear between the concentrations of 0.01 and 100 μg/mL, with a limit of detection at 0.1 ng/g that below the sensory threshold of DDMP (2.06 μg/g). Five cultivars of baked red jujubes from China were evaluated with the method for DDMP content (4.55 ~ 46.50 μg/g). The method based on HS-SPME-GC-MS/MS was validated and was able to determine the DDMP content in cultivars of baked red jujube reliably and sensitively.

1.5. The HS-SPME-GC-MS/MS based method for investigation of effects of baking on distribution of four alkyl pyrazines in red jujube

The alkyl-substituted pyrazine group has been recognized and evaluated to contribute to the aroma of thermally processed food (Fors & Olofsson, 1986). Alkyl -substituted pyrazines were described with with some odor characteristics like nutty, roasted, musty, or sweety in food (Wang et al., 2020). However, the daily intake of methyl pyrazine (7 μg), 2,6-methyl pyrazine (2 μg), trimethyl pyrazine (46 μg), and tetramethyl pyrazine (19 μg) is limited as pyrazine derivatives are proved to have acute and short-term toxicity on rats (Adams et al., 2002). Alkyl-substituted pyrazines are reported to present in raw and processed jujube (Song et al., 2020). Furthermore, thermal processing is considered as a heat-induced condensation of pyrazines ’ formation through the Strecker degradation during the Maillard reaction (Adams et al., 2002). Therefore, in order to monitor the changes of alkyl-substituted pyrazines’ (methyl pyrazine, 2,6-methyl pyrazine, trimethyl pyrazine, and tetramethyl pyrazine) changes in the baking process of red jujube, a method based on HS-SPME-GC-MS/MS has been developed. The calibration curve was constructed in the range of 1–10000 ng/mL with linear correlation coefficients of 0.9947~0.9988. The tracking of the four alkyl-substituted pyrazines in raw and baked red jujube showed the content of methyl pyrazine, 2,6-methyl pyrazine, trimethyl pyrazine, and tetramethyl pyrazine was in the range of 42.25 ~ 916 μg/g, 43.83~143.12 μg/g, 43.83~143.12 μg/g, and 8.37~234.77 μg/g, separately. For raw red jujube, tetramethyl pyrazine was the highest, accounting for 38.71%~59.50% of the total pyrazine. On the other hand, methylpyrazine ranked the highest portion of alkyl pyrazine in baked red jujube with the data of 35.52%~49.76%. The degradation of pyrazines might be correlated with the processes of photocatalytic and biological degradation, oxidative degradation, reductive degradation, and channelization (VRajini, Aparna, Sasikala, & Ramana, 2011).

2. Conclusion and perspectives

The general objective of this research was to get a comprehensive investigation on targeted and untargeted compounds for raw and baked red jujube. The geographical differentiation of aroma profile for winter jujube, the cultivar based aroma characteristics for red jujube in the biggest production area in Xinjiang Province, China, and the targeted compounds with specific odor contributions like DDMP and alkyl pyrazines were evaluated to obtain more information on jujube aroma.

In accordance to achieve this objective, a series of analytical methods have been applied, from traditional methods (GC-MS, E-nose, GC-MS-O, sensory evaluation, omission and recombination experiments) to some innovative techniques (GC-IMS, GC-MS/MS), statistical tools were also relevant (PCA, heatmap, correlation analysis, VIP scores, etc.).

According to the previous studies, the volatiles of fresh jujube were composed by alcohols, acids, aldehydes, esters, ketones, and furans (Chen et al., 2018; Song et al., 2019). Aldehydes (54-78%) and acids (8-26%) were detected as the main volatiles of red jujube (cv. Muzao, Yuanzao, Lizao, Xiangzao, Banzao, Jinsixiaozao, Pingdingdazao, Lingwuchangzao, and Junzao) (Chen et al., 2018; Song et al., 2019). The study also showed that in winter jujube that cultivated in Shandong Province, China, showed that aldehydes were the most dominant volatiles (57%), followed by acids (28%), and esters (12%) (Yang et al., 2019). The aroma characteristics of red jujube (cv. Tangzao; Muzao; Lizao; Qingrunhongzao; Jinsixiaozao; Youzao; and Yuzao from the production areas of Hebei, Shaanxi, and Xinjiang Province, China; cv. 'GAL', 'MSI', and 'PSI' from province of Alicante, Spain) showed the presence of of (E)-2-hexenal, (E)-2-heptenal, (E)-2-octenal, hexanal, 2,3-butanedione, *β*-damascenone, ethyl hexanoate, 3-mercaptohexyl acetate, and 2,5-dimethylpyrazine as the key odor active compounds of the red jujubes (Wang et al., 2018; Zhu &Xiao, 2018; Wojdyło et al., 2016). Besides, the sensory analysis highlighted the attributes of sweet and jujube ID were the strongest sensory attributes. For the processing of jujube, many studies have explored the influence of drying methods (50~95 °C) on the aroma of red jujube (Song et al., 2020; Wang et al., 2016). However, the aroma of thermal processing of red jujube associated with bread or cake has not been revealed yet.

Untargeted and targeted aroma profile has been relatively comprehensively studied in this thesis, further questions were arousing such as the formation pathway of DDMP and how to modulate the formation due to the fact that it may have a negative effect on human health.

HS-GC-IMS, E-nose, and HS-SPME-GC-MS had been applied to analyze the volatile composition of winter jujube from different regions. Benzyl alcohol, octanoic acid, 2-hexenal, linalool, 2-nonenal, and ethyl decanoate were the most common compounds presented in all jujubes. HS-SPME-GC-MS, E-nose, and sensory evaluation were applied for the characterization of the VOCs for red jujube from Xinjiang Province, China. The presence of acetoin, E-2-hexanol, hexanal, acetic acid, and ethyl acetate played an important role in the classification results for six red jujube cultivars from Xinjiang Province. HS-SPME-GC-MS, GC-MS-O, and molecular sensory sciences had been used to track the changes of the key odor active compounds in the baking process of red jujube. *β*-damascenone, 5-butyltetrahydro-2-furanone, and hexanoic acid were the common key odor active compounds in raw and baked red jujube. However, the baking process triggered the formation of aromatic hydrocarbon molecules in red jujube including 1H-pyrrole-2-carboxaldehyde (0.210 μg/g), 2-hexylthiophene (0.011 μg/g), and 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (0.320 μ g/g), especially 5-methyl-2-furancarboxaldehyde (0.360 μg/g) and benzaldehyde (0.110 μg/g) that contributed the most to the caramel-like and roasted aroma of baked red jujube, respectively. HS-SPME-GC-MS/MS had been applied successfully for the detection of DDMP and four alkyl pyrazines in raw and baked red jujube. Five cultivars of baked red jujubes from China were evaluated with the method for DDMP content (4.55 ~ 46.50 μg/g). The results indicated that jujube cultivars with a higher molar ratio of proline to glucose, lower moisture content, and stronger ability to resist water stress should be avoided from baking to modulate the formation of DDMP. Besides, trimethylpyrazine, which accounted for 38.71% ~ 59.50% of the total pyrazine, was the main alkyl pyrazine in raw red jujube. Methylpyrazine accounted for 35.52% ~ 49.76%, the highest portion of alkyl pyrazine in baked red jujube.

The study complemented the study of the aroma profile of jujube (winter jujube, red jujube, and baked red jujube). The common and specific VOCs of winter jujube from different regions were clarified. But the key odor active compounds of winter jujube still needed to be studied in the future. The characteristics of red jujube from Xinjiang Province had been proposed, which would assist in the explanation the Xinjiang Province's regional advantages for red jujubes combining with other chemical compositions and climatic conditions. The definition of the key odor active compounds' changes helped understanding the VOCs that played an important role in the baking arose aroma, which would help the development and research of artificially baked red jujube aroma. The changes of DDMP and alkyl pyrazines had been studied. The correlation analysis indicated that jujube cultivars with a higher molar ratio of proline to glucose, lower moisture content, and stronger ability to resist water stress should be avoided from baking to modulate the formation of DDMP. As DDMP may play an effect on human health, it is necessary to find its formation pathway in red jujube, thus modulating its concentration for health consideration.

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Appendix - publications

1. Articles

(1) **Qiao, Y.**, Bi, J., Chen, Q., Wu, X., Gou, M., Hou, H., Jin, X., and Purcaro, G. **(2021)**. Volatile Profile Characterization of Winter Jujube from Different Regions via HS-SPME-GC/MS and GC-IMS. ***Journal of Food Quality***. https://doi.org/10.1155/2021/9958414.

(2) **Qiao, Y.**, Chen, Q., Bi, J., Wu, X., Jin, X., Gou, M., Yang, X., Purcaro, G. **(2022)**. Investigation of the Volatile Profile of Red Jujube by Using GC-IMS, Multivariate Data Analysis, and Descriptive Sensory Analysis. ***Foods***, 11(3), 421.

(3) **Qiao, Y.**, Bi, J., Chen, Q., Wu, X., Jin, X., Gou, M., Yang, X., Purcaro, G. **(2022)**. Rapid and sensitive quantitation of DDMP (2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one) in baked red jujubes by HS-SPME-GC-MS/MS. ***Food Control***, 108820.

(4) Song, J., Chen, Q., Bi, J., Meng, X., Wu, X., **Qiao, Y.**, Lyu, Y. **(2020)**. GC/MS coupled with MOS e-nose and flash GC e-nose for volatile characterization of Chinese jujubes as affected by different drying methods. ***Food Chemistry***, 331, 127201.

(5) Hou, H., Chen, Q., Bi, J., Wu, X., Jin, X., Li, X., **Qiao, Y.**, Lyu, Y. **(2020)**. Understanding appearance quality improvement of jujube slices during heat pump drying via water state and glass transition. ***Journal of Food Engineering***, 272, 109874.

(6) Hou, H., Chen, Q., Bi, J., Bhandari, B., Wu, X., Jin, X., Shi, Y., **Qiao, Y.**, Gou, M., Shi, J. **(2021)**. Glass transition and crystallization of solid model system of jujube slice as influenced by sugars and organic acids. ***Food Chemistry***, 359, 129935.

(7) Gou, M., Bi, J., Chen, Q., Wu, X., Fauconnier, M. L., **Qiao, Y**. **(2021)**. Advances and Perspectives in Fruits and Vegetables Flavor Based on Molecular Sensory Science. ***Food Reviews International***, 1-14.

(8) **Qiao, Y**., Chen, Q., Gou, M., Zhang, J., Purcaro, G., Jin, X., Wu, X., Lyu, J., and Bi, J. (2022). Elucidation of Baking Induced Changes in Key Odorants of Red Jujube Using Gas Chromatography-Olfactometry, Flavor Dilution Factors, Odor Activity Values, Recombination, and Omission Analysis. ***Intended journal: Journal of Agricultural and Food Chemistry.*** Submitted to Journal.

2. Conference presentation

(1) 6th Food Drying Conference & 7th Symposium for Space Nutrition and Food Engineering. 28/11/2020.