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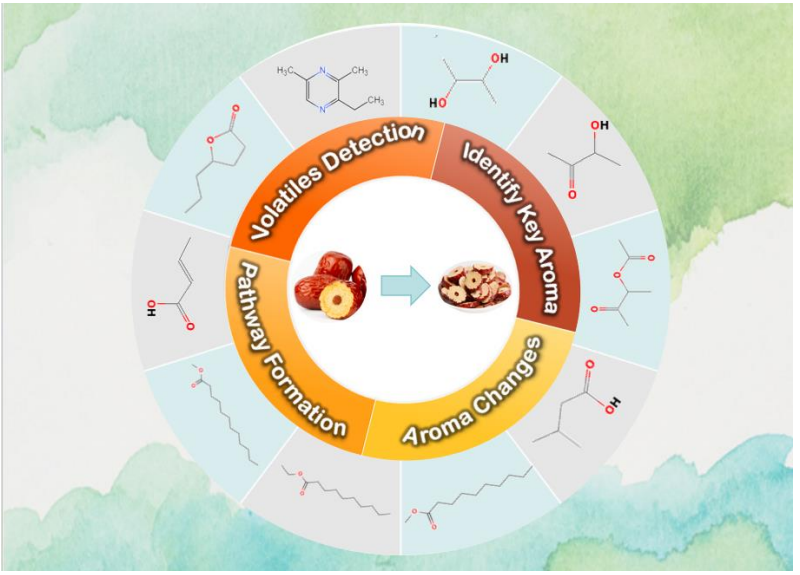
RESEARCH ON THE CHANGES AND REGULATION MECHANISM OF KEY AROMA-ACTIVE COMPOUNDS DURING FREEZE DRYING PROCESS OF *ZIZIPHUS JUJUBA* CV. HUIZAO



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UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

Research on the changes and regulation mechanism of key aroma-active compounds during freeze drying process of *Ziziphus jujuba* cv. Huizao

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Résumé

Min Gou. (2023). "Recherche sur les changements et le mécanisme de régulation des composés aromatiques clés lors du processus de lyophilisation du *Ziziphus jujuba* cv. Huizao" (Thèse de doctorat en anglais). Gembloux, Belgique, Gembloux Agro-Bio Tech, Université de Liège, 213 pages, 18 tableaux, 25 figures.

Résumé :

Le jujube rouge est connu pour son arôme agréable, cependant, bien peu de recherches ont été consacrées à ses arômes clés. La lyophilisation du jujube rouge, une collation appréciée des consommateurs, n'a pas encore fait l'objet d'études approfondies quant à ses caractéristiques aromatiques et aux modifications de son arôme lors de la lyophilisation à l'échelle pilote. Par ailleurs, les effets d'autres méthodes de traitement technologiques classiques sur l'arôme du jujube rouge ont rarement été explorés. En outre, le mécanisme de formation de l'arôme après le traitement reste largement méconnu.

Dans un premier temps, 43 composés volatils ont été identifiés dans le "Huizao". Parmi eux, la 3-hydroxy-2-butanone, la butane-2,3-dione, le méthyl décanoate, l'éthyl décanoate, le méthyl dodécanoate, la 5-propyloxolan-2-one, la 5-butyloxolan-2-one, le 3,5-EDMP, l'acide (E)-but-2-énoïque, l'acide hexanoïque, l'hexanal, le 6-méthyl-5-heptène-2-one, l'acétate de 3-oxobutan-2-yle, le 5-éthyloxolan-2-one et l'acide 3-méthyl-butanoïque ont été identifiés comme des composés aromatiques clés selon une évaluation sensorielle. Ces composés contribuent aux notes vertes, sucrées, florales, fruitées, crémeuses, aigres/rances et de noix du "Huizao". De plus, 17 composés volatils liés aux glycosides ont été détectés après hydrolyse enzymatique par la β-glucosidase.

Les composés aromatiques du "Huizao" présents après lyophilisation à l'échelle pilote ont été analysés par GC-O-MS. La teneur totale en composés aromatiques a diminué de 26,71 % par rapport au jujube rouge brut. Les teneurs en cétones et en acides ont diminué de 63,33 % et 62,88 %, tandis que les teneurs en esters, en lactones et en alcools ont augmenté de 34,10 %, 8,52 % et 480,17 %, respectivement. De plus, 14 composés aromatiques clés

ont été identifiés dans le "Huizao" lyophilisé sur base d'une analyse sensorielle. Parmi eux, le 3,5-EDMP a présenté la plus forte OAV (2 687) et a dominé le profil aromatique avec des notes grillées. De plus, l'éthyl heptanoate, l'acétate d'hexyle, le 2,3-butanediol, l'éthyl dodécanoate et le 5-heptyloxolan-2-one ont été identifiés comme des composés aromatiques clés dans le "Huizao" lyophilisé et ce pour la première fois.

Une analyse en réseau a révélé des corrélations significatives entre la sérine, la glycine, la proline, la valine, la cystéine, l'arginine, l'acide glutamique, la lysine et la leucine avec les pyrazines, dominant la note grillée du "Huizao" lyophilisé. L'acide linoléique, l'acide α-linolénique et l'acide oléique, en corrélation avec l'activité de la lipoxygénase, ont participé à l'augmentation de la concentration en esters (de 412 à 9 486 μg/kg), contribuant aux notes fruitées et sucrées du "Huizao" lyophilisé. De plus, selon le test de Mantel, l'influence des facteurs sur la formation de l'arôme du "Huizao" lyophilisé a été classée comme suit : température > activité enzymatique > acides gras > acides aminés.

Finalement, la formation des alkylpyrazines, basée une matrice de type jujube rouge dans les conditions de la lyophilisation, a été étudiée. Des modèles à l'état solide avec différents pH ont été établis, les composés volatils ont été détectés au cours du processus de réaction, et la corrélation entre ces composés a également été analysée. La teneur en pyrazines dans le modèle à pH 7,8 était seulement de 11,34 % plus élevée que dans le modèle à pH 5,5. Dans le modèle à pH 5,5, la butane-2,3-dione, la pentane-2,3-dione, l'acide pyruvique, l'isopropanol, l'acétone et l'acide 2-hydroxypropionique présentaient une corrélation significative avec le 3,5-EDMP. Dans la matrice de jujube rouge, la formation majoritaire du 3,5-EDMP résulte de la condensation de la 2-aminopentan-3-one produite par le pentane-2,3-dione et de l'aminacétone produite par la dégradation de Strecker du méthylglyoxal, formant ainsi la dihydropyrazine, qui est ensuite oxydée pour produire le 3,5-EDMP.

Enfin, l'effet, sur l'arôme du jujube rouge, d'autres méthodes de traitement technologiques classiques a été étudié par GC-MS/MS, GC-O, OAV à l'aide d'une analyse descriptive quantitative. Après la lyophilisation, la teneur totale en arômes a augmenté de 0,90 %, tandis qu'elle a diminué de

respectivement 51,59 %, 74,11 % et 78,74 % après la cuisson, la friture et la cuisson à la vapeur. Les esters ont prédominé dans l'arôme, contribuant aux notes sucrées et fruitées des jujubes lyophilisés, en particulier les esters éthyliques. Les aldéhydes, en particulier le (*E*, *E*)-déca-2,4-diénal, domine dans la note grasse des échantillons frits. Les pyrazines prédominent dans les notes grillées des échantillons cuits au four et cela suite à la réaction de Maillard.

Mots clés : jujube rouge, lyophilisation, traitement, composés aromatiques clés, HS-SPME-GC-MS/O, analyse de corrélation, alkylpyrazine, voie de formation

Abstract

Min Gou. (2023). "Research on the changes and regulation mechanism of key aroma-active compounds during freeze drying process of *Ziziphus jujuba* cv. Huizao" (PhD Dissertation in English). Gembloux, Belgique, Gembloux Agro-Bio Tech, Université de Liège, 213 pages, 18 tables, 25 figures.

Summary:

Red jujube has a pleasant aroma, but there is a lack of research on the key aromas of red jujube. Freeze-dried red jujube, as a kind of leisure food favoured by consumers, has not been studied in terms of its aroma characteristics and changes in aroma during pilot scale freeze drying, and the effects of other typical processing methods on the aroma of red jujube have rarely been studied and lack of research on the mechanism of aroma formation after processing.

Firstly, there were 43 free volatile compounds detected in "Huizao", of which, 3-hydroxy-2-butanone, butane-2,3-dione, methyl decanoate, ethyl decanoate, methyl dodecanoate, 5-propyloxolan-2-one, 5-butyloxolan-2-one, 3,5-EDMP, (*E*)-but-2-enoic acid, hexanoic acid, hexanal, 6-methyl-5-hepten-2-one, 3-oxobutan-2-yl acetate, 5-ethyloxolan-2-one, and 3-methyl-butanoic acid were identified as key aroma-active compounds based on molecular sensory science. These compounds contributed the green, sweet, floral, fruity, cream, sour/rancid and nut notes to "Huizao". Besides, there were 17 glycosidically bound volatile compounds detected after enzymatic hydrolysis by *β*-glucosidase.

Secondly, the aroma compounds of "Huizao" after pilot scale freeze drying were analysed by GC-O-MS. The total aroma compounds content was decreased 26.71% compared with raw red jujube, of which ketones and acids contents were decreased 63.33% and 62.88%, while, the esters, lactones and alcohols contents were increased 34.10%, 8.52% and 480.17%, respectively. In addition, 14 key aroma-active compounds were identification in freeze-dried "Huizao" based on molecular sensory science. In which, 3,5-EDMP had the highest OAV (2,687) and dominated the roasty note of aroma profile. And ethyl heptanoate, hexyl acetate, 2,3-butanediol, ethyl dodecanoate and 5-heptyloxolan-2-one were newly identified as the key aroma-active

compounds in freeze-dried “Huizao”.

Thirdly, through the network analysis, serine, glycine, proline, valine, cysteine, arginine, glutamic acid, lysine and leucine had the significant correlation with pyrazines, dominated the roasty note of freeze-dried “Huizao”. Linoleic acid, α-linolenic acid and oleic acid with lipoxygenase had important effects on the increase of esters (from 412 to 9,486 μg/ kg), contributed fruity and sweet notes of freeze-dried “Huizao”. Besides, through the Mantel test, the influence degree of factors on the formation of freeze-dried “Huizao” aroma was ranked as temperature > enzyme activity > fatty acids > amino acids.

Fourthly, the alkylpyrazines formation based on red jujube matrix and under the condition of freeze drying were investigated, the solid-state models with different pH were established and the volatile compounds were detected during the reaction process, the correlation also be analysed between these compounds. The content of pyrazines in pH 7.8 was only 11.34% higher than pH 5.5 model. And in pH 5.5 model, butane-2,3-dione, pentane-2,3-dione, pyruvic acid, isopropyl alcohol, acetone and 2-hydroxypropionic acid had the significant correlation with 3,5-EDMP. In red jujube matrix, the dominant formation of 3,5-EDMP is condensation of 2-aminopentan-3-one produced by pentane-2,3-dione and aminoacetone produced by the Strecker degradation of methylglyoxal to form dihydropyrazine, which is then oxidised to form 3,5-EDMP.

Finally, the effect of other typical processing on aroma of red jujube were studied GC-MS/MS, GC-O, OAV and quantitative descriptive analysis. After freeze drying, the total aroma content increased by 0.90%, while it decreased by 51.59%, 74.11% and 78.74% after baking, frying and steaming, respectively. Esters dominated the aroma and contributed sweet and fruity notes in freeze-dried jujubes, especially ethyl esters. Aldehydes, especially (*E, E*)-deca-2,4-dienal dominated the fatty note in fried samples. Pyrazines dominated the roasty notes of baked samples due to the Maillard reaction.

Keywords: red jujube, freeze drying, processing, key aroma-active compounds, HS-SPME-GC-MS/O, correlation analysis, alkylpyrazine, formation pathway

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Min Gou

03/10/2023 in Gembloux, Belgium

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List of Abbreviations

FD, Freeze drying

SPME, Solid-phase microextraction

PA, Polyacrylate

PDMS, Poly-dimethylsiloxane

CAR, Carboxen

DVB, Divinylbenzene

GC-MS, Gas chromatography–mass spectrometry

GC-O, Gas chromatography−olfactometry

OAV, Odour activity value

GC, Gas chromatography

MS, Mass spectrometry

AEDA, Aroma extract dilution analysis

FD value, Dilution factor value

DFA, Detection frequency analysis

OSME, Direct intensity analysis

FID, Flame ionization detector

QDA, Quantitative descriptive analysis

GC × GC - MS, Two-dimensional gas chromatography combined with mass spectrometry

ADH, Alcohol dehydrogenase

AAT, Alcohol acyltransferase

PDC, pyruvate decarboxylase

LOX, Lipoxygenase

HPL, Hydroperoxide lyase

ACX, Acetyl-CoA oxidase

PDMS/DVB, Polydimethylsiloxane-divinnylbenzene

HS-SPME, Headspace solid-phase microextraction

SD, Standard deviation

GC-O-MS, Gas chromatography-olfactometry-mass spectrometry

LRI, Linear retention indices

FDJ, Freeze-dried jujube

DTT, Dithiothreitol

PVPP, Crosslinked polyvinylpyrrolidone

NADH, Nicotinamide adenine dinucleotide

DTNB, 5,5'-Dithiobis-(2-nitrobenzoic acid)

Acetyl CoA, Acetoacetyl coenzyme A

RI, Retention indices

C12:0, Lauric acid

C14:0, Myristic acid

C14:1n5, Myristoleic acid

C16:0, Palmitic acid

C16:1n7, Palmitoleic acid

C18:0, Stearic acid

C18:1n9c, Oleic acid

C18:2n6c, Linoleic acid

C18:3n3, α-Linolenic acid

FAAs, Free amino acids

Gly, Glycine

Val, Valine

His, Histidine

Lys, Lysine

Leu, Leucine

Cysthi, Cystathionine

EOHNH2, Ethanolamine

Ser, Serine

Arg, Arginine

β-AiBA, β-Aminoisobutyric acid

γ-ABA, γ-Aminobutyric acid

Asp, Aspartic acid

Thr, Threonine

Ala, Alanine

Phe, Phenylalanine

α-AAA, *α*-Aminoadipic acid

Cys, Cysteine

Pro, Proline

Tyr, Tyrosine

Ser, Serine

Hypro, Hydroxyproline

Met, Methionine

Glu, Glutamic acid

Cit, Citrulline

PEA, *o*-Phosphoethanolamine

Tau, Taurine

PCA, Principal component analysis

FFAs, Free fatty acids

GC- IMS, Gas chromatography-ion mobility spectrometry

GC-MS/MS, Gas chromatography - tandem mass spectrometry

PDMS/DVB/CAR, Polydimethylsiloxane-divinylbenzene

HPLC-MS/MS, High performance liquid chromatography - tandem mass spectrometry

QC, Quality control sample

3,5-EDMP, 2-Ethyl-3,5-dimethyl-pyrazine

VIP, Variable importance in projection

DDMP, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4(H)-Pyran-4-One

1-DG,1-Deoxyglucosone

5-HMF, 5-(Hydroxymethyl)furfural

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Chapter Ⅰ. General Introduction

1. Background

Red jujube (*Zizyphus jujuba* Mill.) belongs to the family Rhamnaceae, is indigenous in China with a long history above 4000 years and widely consumed in more than 30 countries around the world (Chen et al., 2014; Gou, et al., 2023). There are more than 700 cultivars of red jujube in China, among which there are more edible and dried varieties. Among them, *Ziziphus jujuba* cv. Huizao is one of the most popular cultivars by consumers, not only has a rich nutritional value, but also has a unique aroma.

Aroma has a significant effect on the overall quality of food, which is a complex mixture of volatile compounds. Only a part of these volatile compounds could contribute special aroma to the food, making it play a major role in the presentation of the aroma characteristics of the food. Volatile compounds with this property are called key aroma or aroma active compounds. Recently, most studies on the aroma of red jujube focused on the differences between cultivars and regions, as well as the aroma after processing (Hernández et al., 2016; Qiao et al., 2021; Qiao, Bi, et al., 2022; Qiao, Chen, et al., 2022; J. Song et al., 2019, 2020; Xin Sun et al., 2019a). There are few studies on characteristic aroma of red jujube. According reports, aldehydes, acids and esters were dominant volatile compounds in red jujube (Huang et al., 2021; S. Liu et al., 2015a). In addition to free volatile compounds, glycosidically bound volatile compounds are also exist in food, which are potential source of aroma. Aroma enhancement can be achieved for products by enzymatic hydrolysis of glycosidically bound aroma compounds.

Processing is a common means of changing the aroma of food. For red jujube, hot air drying, heat pump drying, freeze drying, instant controlled pressure drop drying, baking, steaming, frying and fermentation are common processing methods. With development of freeze drying, freeze-dried red jujube become more and more popular. Freeze-dried product showed the better performance in nutrient retention, colour and shape. However, the aroma of freeze-dried product showed different degrees of loss (37.5% to 97%) (Chin et al., 2008; Dimelow et al., 2005; Feng et al., 2021; Jeyaprakash et al., 2020; Mui et al., 2002; Rajkumar et al., 2017a; J. Zhang et al., 2019a). In these researches, the freeze-dried machine was usually applied based on the laboratory scale, and the temperature usually set below 30 °C, which is quite different from the actual production in the industry. In the industry scale freeze drying, the multi-stage and variable-temperature procedure was applied with a

higher temperature of heating plate (85~65 °C), the temperature of the material will gradually increase from the freezing temperature (-40 °C) to the heating plate temperature (65 °C). Under this temperature condition, the chemical reaction will occur to enhance the aroma of products. As other typical processing methods for red jujube, baking, frying and steaming had significant difference with freeze drying, and they also have obvious influence on aroma of red jujube.

2.Objective

(1) to comprehensively evaluated the overall aroma of red jujube (cv. Huizao) and identify the key aroma-active compounds in red jujube.

(2) to understand how the jujube aroma was modified after freeze drying and identify the key aroma-active compounds in freeze-dried red jujube (cv. Huizao)

(3) to reveal the changes in aroma, aroma precursors and related enzyme activities in the freeze drying process of red jujube (cv. Huizao) and analyse their correlation.

(4) to reveal the formation pathway of key aroma compounds during freeze drying based on real red jujube model system.

(5) to investigate the effect of typical processing methods on aroma of red jujube (cv. Huizao) and predict the formation pathway of key aroma compounds in different processing methods.

3.Research outline

The literature review mainly introduces the work undertaken in this thesis, included describing the red jujube and its products, the research progress of aroma analysis methods and aroma of red jujube and their products, and the effect of processing methods on aroma formation in red jujube (Chapter II).

The research content of the thesis work is shown in **Figure 1-1**. Firstly, the overall aroma profile of red jujube (cv. Huizao) was comprehensively evaluated from both free and glycosidically bound volatile compounds. Moreover, the key aroma-active compounds of red jujube were further identified based on molecular sensory science technology, including GC-MS, GC-O, aroma recombination and omission test (Chapter III).

Secondly, the effect of pilot scale freeze drying on aroma profile of red jujube (cv. Huizao) was first analysed, and key aroma-active compounds of freeze-dried red

jujube were further identified based on molecular sensory science and technology (Chapter IV).

Thirdly, the changes of aroma, sugars, fatty acid and free amino acids, and related enzyme activities in the pilot scale freeze drying process of red jujube (cv. Huizao) were investigated; and the correlation between aroma and aroma precursors and enzyme activities, main precursors of aroma-active compounds were analysed through the Mantel test and network analysis (Chapter V).

Fourthly, this study intends to use a solid-state model system, and the react condition will apply the highest commercial plate temperature (80 °C) and processing time (10 h) to study the formation pathway of alkylpyrazines in freeze-dried red jujube crisps (Chapter VI).

Fifthly, the effect of typical processing methods, including steaming, freeze drying, frying and baking, on aroma on aroma of red jujube (cv. Huizao) were studied via sensory evaluation, GC-O, GC-MS/MS and OAV. Furthermore, the formation pathway of key aroma compounds in different processing methods was explored via untargeted metabolomics (Chapter VII).

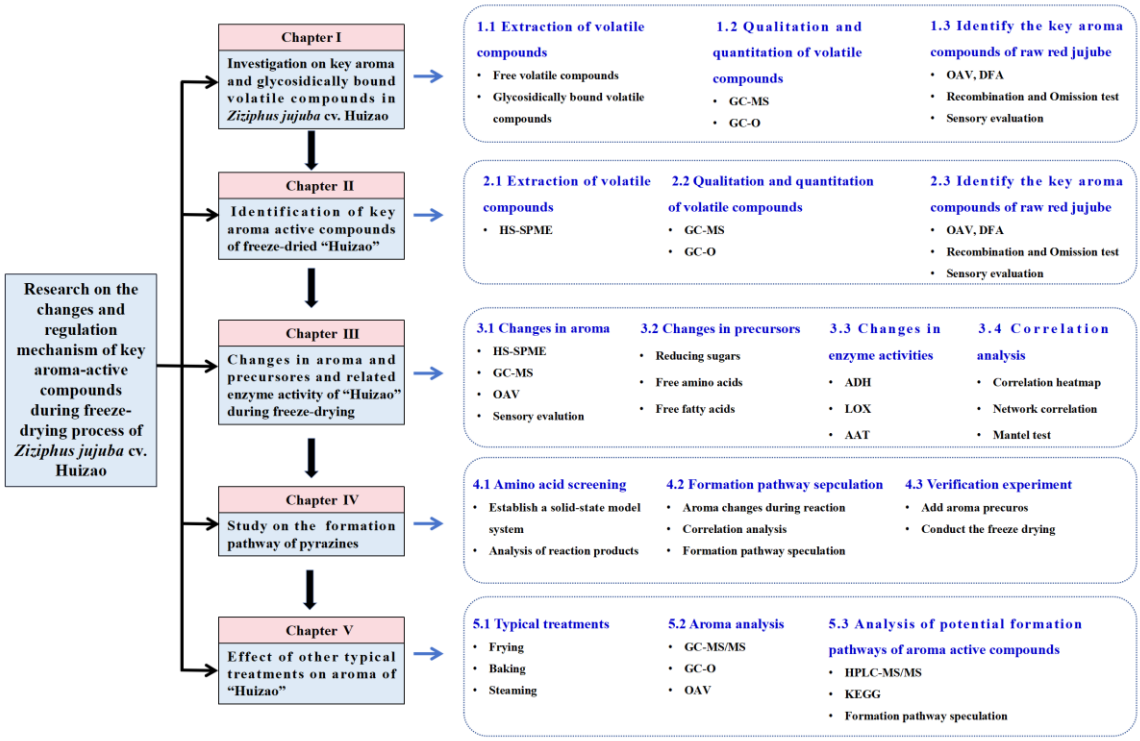

Figure 1-1 Technical route of the research content.

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Chapter II. Literature review on red jujube and its aroma

Abstract: Red jujube (*Ziziphus jujuba* Mill.) is a homology of food and medicine, which is not only rich in nutrition, but also has a pleasant aroma. In addition to edible red jujube, processed red jujube products are also favoured by consumers. This review gives an overview of red jujube and its products, the formation of red jujube aroma and the influencing factors affecting the aroma of red jujube. In addition, the aroma extraction and analysis techniques that will be used in this thesis are also introduced. In addition, it also introduces the progress of aroma research on red jujube and its products in recent years.

Keywords: red jujube, aroma extraction, aroma analysis, processing

1. Introduction

1.1. Red jujube and its products

Red jujube (*Ziziphus jujuba* Mill.) is a plant of the family Rhamnaceae, which has a long history in China (Song et al., 2019). China's red jujube production accounts for about 90% of the world, and there are about 700 cultivars (Guo et al., 2010). Huizao (*Ziziphus jujuba* cv. Huizao) is a high-quality cultivar. Huizao in Xinjiang was introduced from Xinzheng, Henan Province in the early 1970s (Niu et al., 2009). Huizao has a dense texture, high content of sugar, low content of water and pleasant flavour. In addition, it contains many active ingredients, such as poly-saccharides, phenols, saponins, cyclic nucleotides, alkaloids, triterpenes, sterols, essential oils, etc, is popular in consumers (Chen et al., 2014; Yuxing Liu et al., 2021; Qiao, Chen, et al., 2022; Xuan et al., 2021).

Traditionally, raw red jujube as the main sales form, and the sales of roughly processed and deep-processed products only account for about 20% of the total sales of red jujube, and the degree of red jujube resource utilization is low (Yao & Yin, 2006). Most of red jujube is consumed by eaten fresh or cooked by steaming or boiling. In recent years, with the continuous improvement of the processing level, some new types of red jujube products have been gradually developed, such as jujube juice, jujube wine, jujube vinegar, jujube milk, jujube tea, jujube crisps, jujube cake, powdered jujube, candied snacks (jam, jelly, and pickles) and fried jujube, etc. Common processing methods of red jujube mainly include hot air drying, heat pump drying, baking, freeze drying, fermentation and others. Freeze-dried red jujube is the most popular of these processed products because of its better nutrition, appearance, colour and flavour.

1.2. Aroma formation of red jujube

Most of volatile compounds in red jujube are belong to secondary metabolites, such as alcohols, aldehydes, acids, ketones and esters. These compounds can be formed from amino acids, fatty acids and sugars through the action of a series of enzymes during the growth and maturity of red jujube (oxygenases, dehydrogenases, lyases, and transferases, etc.).

1.2.1 Biosynthesis of sugars as precursors

Sugar is a precursor for the metabolic synthesis of alcohols, acids, esters. During anaerobic respiration, monosaccharides are converted to pyruvate, which is

catalyzed by dehydrogenases to form acetyl-CoA and further ester compounds (El Hadi et al., 2013; Schwab et al., 2008a).

1.2.2 Biosynthesis of amino acids as precursors

Fruits aroma compounds contain branched chain aliphatic alcohols, aldehydes, ketones, and esters, which are mainly derived from amino acid metabolism (**Figure 2-1**). The aliphatic amino acids involved in the synthesis of aroma compounds are mainly valine, leucine, isoleucine, alanine, and cysteine; the aromatic amino acids are tyrosine, tryptophan, and phenylpropyl from shikimic acid (Wendakoon et al., 2004). The enzyme activity and substrate specificity in the amino acid metabolism pathway determine the type and content of branched alcohols and esters (Brückner & Wyllie, 2008). The key enzymes involved in the synthesis of volatile compounds using amino acids as precursors are alcohol dehydrogenase (ADH), alcohol acyltransferase (AAT), and pyruvate decarboxylase (PDC) (El Hadi et al., 2013). The aroma of strawberry is the amino acid under the action of transaminase and pyruvate dehydrocarboxylase, part of which is generated by the transamination and decarboxylation reaction, and the other is produced by the conversion of alanine to alcohols and esters (Gonda, Bar, Portnoy, Lev, Burger, Schaffer, Tadmor, Gepstein, Giovannoni, Katzir, et al., 2010).

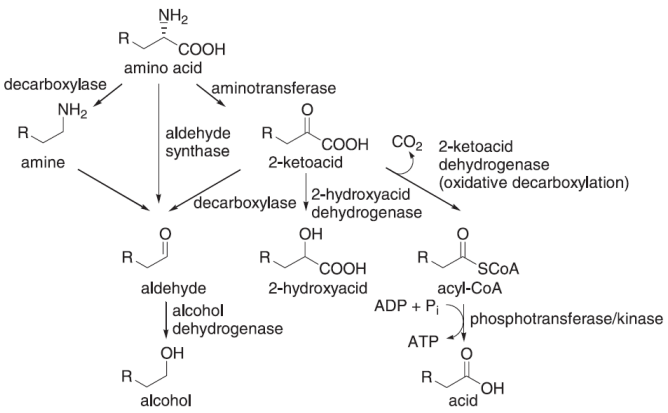


Figure 2-1 The biosynthetic pathway for amino acid degradation to volatiles (Schwab et al., 2008).

1.2.3 Biosynthesis of fatty acids as precursors

Fatty acids are the main precursors that form the aroma compounds of food. The linear fatty alcohols, aldehydes, ketones, and esters in the aroma components are

mainly derived from the oxidation of fatty acids (El Hadi et al., 2013). The fatty acid pathway has two branches, LOX oxidation and *β*-oxidation (**Figure 2-2**) (El Hadi et al., 2013). (1) LOX oxidation is the first step in catalyzing the synthesis of aromatic substances, and the precursor materials without volatile substances are converted into products with food flavour. The LOX pathway uses linoleic acid and linolenic acid as substrates to form hydroperoxides. Hydroperoxides form hexanal or hexenal under the action of hydroperoxide lyase (HPL). Aldehydes are produced by alcohol dehydrogenase (ADH) to form the corresponding alcohol, and alcohol to form the corresponding ester under the action of alcohol acyltransferase (AAT); (2) *β*-oxidation process produces acetic acid, butyric acid and hexanoic acid, and then reduced to the corresponding alcohols. These alcohols form esters under the action of acyl-CoA and alcohol acyltransferases (Sanz & Pérez, 2010), or they can first form anti-2-ketoacyl-CoAs under the action of acetyl-CoA oxidase (ACX) A, followed by the formation of lactones by some enzymes (Xi et al., 2012a).

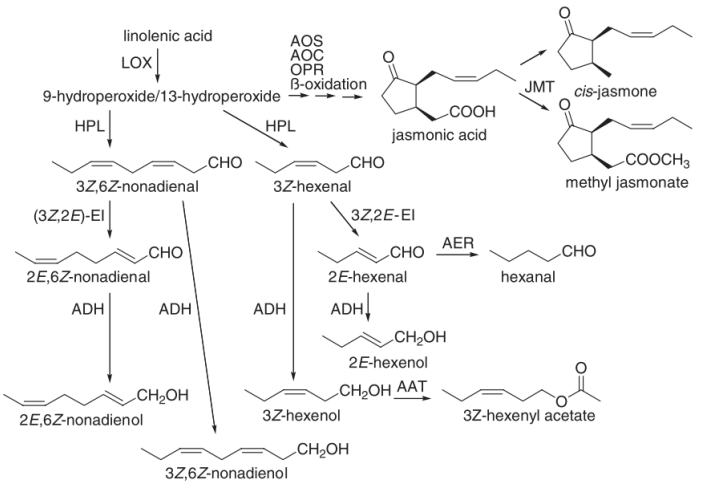


Figure 2-2 The biosynthetic pathway for linolenic acid degradation to volatiles (El Hadi et al., 2013).

AAT, alcohol acyl CoA transferase; ADH, alcohol dehydrogenase; AER, alkenal oxidoreductase; AOC, allene oxide cyclase; AOS, allene oxide synthase; HPL, hydroperoxide lyase; JMT, jasmonate methyltransferase; LOX, lipoxygenase; OPR, 12-oxo-phytodienoic acid reductase; 3Z,2E-EI, 3Z,2E-enal isomerase.

1.3. Effect of processing on aroma formation of red jujube

Processing, such as drying, can contribute a new flavour to red jujube. Due to the influence of different temperatures, heat transfer methods and heating media in the

process, red jujube will undergo Maillard reactions, lipid oxidation, amino acid degradation, and their combined reactions, resulting in significant differences in the aroma and quality of the products (Wei et al., 2023; Zhou et al., 2022). Maillard reaction is an important way of food flavour formation, which has a direct impact on the nutritional quality, safety, sensory characteristics and consumer acceptance of food. Reducing sugar and amino acids can react Maillard reaction at high temperature, which is an important way to form the characteristic flavour of dried red jujube products.

Lipid oxidation is another way to form flavour during the processing. Unsaturated fatty acids in the double bond through auto-oxidation, photo-oxidation or enzymatic oxidation of three ways to generate hydroperoxides, hydroperoxides can be polymerised to form polymers, dehydration to form ketones, acid esters, or continue to oxidation to generate secondary oxidation products, secondary oxidation products can also be decomposed to generate aldehydes, ketones, alcohols, hydrocarbons, carboxylic acids, esters, furans, and a series of small molecules, such as ester compounds. These substances may continue to interact with the intermediate products of the Maillard reaction to form pyrazines, thiazoles, pyridines, sulphur-containing volatile compounds and so on (Wei et al., 2023)(Jian Zhao et al., 2019).

Jujube wine, jujube vinegar, jujube yogurt and fermented jujube juice with unique flavour and rich nutrition can be produced after fermentation. The production of flavour substances through fermentation is a relatively complex process. Microorganisms such as yeast, acetobacteria and Lactobacillus plantarum produce primary metabolites (such as ethanol and acetic acid) by metabolizing sugars in jujube. In the post-maturing stage, complex biochemical reactions will also occur to produce a large number of secondary metabolites, such as esters, aldehydes, ketones and alcohols.The characteristic flavours of jujube wine and jujube vinegar are composed of these secondary metabolites, mainly esters (Xin Sun et al., 2019a).

1.4. Extraction methods of volatile compounds

1.4.1 Solid-phase microextraction (SPME)

SPME is a non-solvent extraction technology developed by Professor Pawliszyn and colleagues of the University of Waterloo in Canada based on solid-phase extraction in the 1990s (Kataoka et al., 2000). SPME is a simple, fast and efficient sample preparation method. It could integrate sampling, extraction, concentration and sample injection into one step. It is currently the most widely used extraction

method (Zhang et al., 2016).

SPME uses a fused silica fiber (1 or 2 cm in length) to concentrate the volatiles in the sample. Aroma substances would be analyzed and adsorbed on specific types of fibers according to their own characteristics. Usually the choice of fiber type depends on the properties of the volatile compounds to be extracted (Portillo-Castillo et al., 2018). Currently, polyacrylate (PA), poly-dimethylsiloxane (PDMS) and mixed-phase sorbents (CAR (carboxen) / PDMS， PDMS / DVB (divinylbenzene) and DVB / CAR / PDMS) were commonly used fibers in literatures. Among these fibers, PDMS has a high approximation for the extraction of non-polar compounds. The PA is suitable for extracting polar compounds (Portillo-Castillo et al., 2018). Mixed-phase sorbents could be applied for extracting more lower molecular-mass volatiles and more polar compounds due to their lager specific surface areas (Kataoka & Saito, 2011). Among these mixed-phase coating fiber, PDMS/DVB and DVB/CAR/PDMS are bipolar, that is, both non-polar and some polar compounds could be extracted (Portillo-Castillo et al., 2018). To obtain a more complete aroma composition, PDMS / DVB and DVB / CAR / PDMS are commonly used as both polar and non-polar compounds were existed in aroma of fruits and vegetables. Maggi et al. identified a series of volatile eight-carbon alcohol compounds in mushrooms by HS-SPME-GC-MS using a PDMS fiber and the major volatile compound was 1-octen-3-ol (Maggi et al., 2010). A literature reported PA fiber could extract more target flavour volatiles than the commonly used PDMS fiber in fruit juices (Kataoka et al., 2000). Volatile compounds in several fruits such as papaya (Pino & A., 2014), melon (Lignou et al., 2013), peach (Montero-Prado et al., 2013), kiwi (Sarbu et al., 2012), apple (Aprea et al., 2012), pear (Qin et al., 2012), mango (Fan et al., 2010) and banana (Heliofabia et al., 2012) were also analyzed using DVB/CAR/PDMS fiber.

1.4.2 Extraction of bound (glycoside) volatile compounds

In fruits and vegetables, volatiles can be present in free and bound (glycoside) form. The bound volatiles are considered a potential source of aroma compounds. As we all know, the glycoside-bound volatile content is usually 10:1 higher than the free volatile. Glycoside-bound aroma compounds have been reported in many fruits and vegetables, such as kiwifruit, citrus, grape, pineapple, lemon, cantaloupe, and celery et al. Generally, the free volatiles could be extracted directly, but bound volatiles need some pre-treatment. The bound volatiles need to be separated by C18

column or Amberlite XAD-2 resins, then isolation would be hydrolyzed with Rapidase AR2000 enzyme or acid, finally, the released volatiles were analyzed using above mentioned free aroma extraction methods (Chen et al., 2020; Nasi et al., 2008; Wu et al., 2020).

1.5. Analysis of volatile compounds

To date, numerous compounds have been identified from the volatile components of foods. Only a part of the volatile components can contribute special aroma to the food, making it play a major role in the presentation of the aroma characteristics of the food. Volatile components with this property are called key odourant or aroma active component.

1.5.1 Molecular sensory sciences

Molecular sensory science technology, which is proposed by Professor Peter Schieberle in 2007 (Steinhaus & Schieberle, 2007), provides a way to identify the key aroma compounds. Molecular sensory science is often based on GC-MS and GC-O, combined with OAV, omission test and aroma reconstitution experiment to qualify, quantify and describe the flavour at the molecular level, and accurately construct the flavour recombination of food to determine flavour composition in food.

1.5.2 GC-MS

The composition of volatile substances in food is complex; hence, the precondition for identification of aroma compounds is that the volatile components could be separated well. At present, gas chromatography (GC) is the main method for separating volatile compounds for its high sensitivity and good separation ability. Because the distribution coefficients of the volatile compounds in the gas phase of the chromatographic column and the stationary phase of different coatings are different (Biniecka & Caroli, 2011). GC separates the mixture mainly accords the polarity, boiling point, and adsorption properties of the sample. Since volatile compounds are composed of multiple compounds, different compounds have different polarities. Usually, both polar and non-polar chromatographic columns are used to extract the aroma comprehensively.

MS is a powerful structural analysis tool, which could provide more information for structural characterization, and is an ideal chromatographic detector (Gross & Todd, 2005). It can detect all compounds that be ionized, and obtain mass spectrum

at each time point which will provide information of molecular structure of compounds and can be used for qualitative and quantitative analysis (Verma & Srivastav, 2020). Since the late 1950s, GC-MS has been widely used as a hyphenated technology that can be used to qualitatively and quantify aroma substances. GC-MS qualitatively analyzes volatile compounds by matching the standard spectrum library, and quantifies the extracted analytes at a certain optimal temperature (Verma & Srivastav, 2020). With the development of computer software and electronic technology, this technology has become widely applied.

Generally, GC-MS is mainly applied to establish the volatile fingerprint and determine the aroma content of fruits and vegetables. Song et al. used GC-MS qualitative and quantitative volatile compounds in Chinese jujubes after different treatments. They found instant controlled pressure drop dried jujube could produce the most diverse aroma profile through NIST11 library and vacuum freeze dried jujube had the highest content of total aroma compounds through internal standard method (Song et al., 2020). Zhang et al. (2018) applied GC-MS combined MS library, standard products, retention index and odour description methods for 3-(methylthio) propanal, 1-octen-3-one and pyrazines identification in dried mushroom. Dou et al. (2020) used GC-MS combined with area normalization method evaluated the aroma quality of banana during different harvest time. Results indicate that banana harvested in March have more volatile aroma compounds. Although the area normalization method could not accurately obtain the content of aroma compounds, it could easily compare the content differences between different samples.

1.5.3 GC-O

GC-O is an effective method for identifying aroma active compounds developed in recent years. GC-O associates the human nose as a detector with gas or mass spectrometry. It can identify key characteristic components that affect the aroma or aroma of the entire food and determine the aroma intensity of odourous substances. The commonly used detection technology in GC-O can be roughly divided into the following 3 types according to different principles and 3 types of GC-O tests would be showed with **Figure 2-3**.

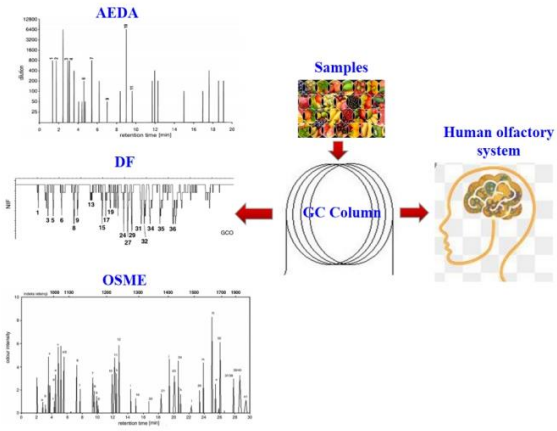


Figure 2-3 Three types diagram of GC-O tests.
AEDA, aroma extract dilution analysis; DF, detection frequency; OSME, direct intensity analysis. Adapted from Plutowska and Wardencki

The dilution to threshold method is a relatively widely used GC-O method. In this method, aroma compounds are gradually diluted. Each diluted concentration sample is subjected to GC-O analysis and sensory evaluation. The group members (usually 8-12) record the times of aroma and aroma properties in the sniffing mouth. The most commonly used methods for dilution to the threshold is AEDA (aroma extraction dilution analysis) (Cheng et al., 2016). In AEDA, panelists evaluate samples with increasing dilution order and the contribution of an aroma active compound is given by its dilution factor value (FD value). The overall results are reported by listing the FD values (Acampora Zellner et al., 2008).

In the DF (detection frequency) fragrance spectrum, the peak height indicates the number of times it is smelled, and has nothing to do with the intensity of the fragrance. There is one common frequency detection method, which is to characterize the fragrance contribution of each substance by the total number of times that each substance is smelled by the scent staff. In the test, substances with a DF ≥ 3 and at least one smell by each of the three evaluators were determined as the aroma active compounds of the test raw materials (Cheng et al., 2013).

The direct intensity method, also called OSME (odour) method, uses a computerized 16-point system to record the change in odour intensity over time and the corresponding odour characteristics, and obtains an OSME spectrum similar to FID detection (Su & Chien, 2010). In the OSME spectrum, the peak of the spectrum

is higher, the odour intensity of the compound and its contribution to the fragrance are greater.

These three GC-O methods have their own advantages and disadvantages. In general, AEDA takes the longest time and requires systematic training of evaluators; similarly, OSME also has higher requirements for evaluators; DF method is the simplest method and the easiest to operate and does not require evaluators to train, but not so accurate in some cases. Therefore, these GC-O methods could be used at the same time to complement each other to obtain accurate and reliable results.

1.5.4 Odour activity value (OAV)

OAV is an effective evaluation for the contribution of aroma compounds in the presentation of aroma characteristics in food substrates. OAV is determined by the concentration of the aroma substance and its threshold value. The calculation formula is as follows: OAV = concentration of aroma substance in the matrix / the threshold value of the substance in the matrix. Generally, the components would be considered to have overall aroma characteristics with an OAV value ≥ 1. And the OAV value greater, its contribution is greater (Cheng et al., 2016). This method has been successfully applied to the identification of key aroma substances in fruits and vegetables, such as apple juice (J. Guo et al., 2020), *terebinth* fruits (Amanpour, Guclu, et al., 2019), and mushroom (X. Xu et al., 2019). In order to explore the consistency of the analysis results of different key aroma identification methods. Zhang et al. (2019) used OAV and DFA GC-O to identify the aroma active components in cantaloupe. The results showed that a total of 42 volatile substances were identified in mango juice, and 6 components were detected only by OAV, while 4 components were detected only by DFA. The two methods are consistent in identifying key aroma components of mango juice, and each has its own characteristics. The OAV method simplifies the analysis of complex aromas in food.

1.5.5 Omission analysis and aroma reconstitution

To further identify the key aroma compounds of food, omission experiments and aroma recombination experiments should be performed after GC-O and OAV. The first step of this method is to establish a synthetic model mixture with a composition and flavour properties are similar with the raw sample or extract. The second step is to operate omission tests on the model mixture. These omission tests could evaluate the contribution of the volatile compound of a sample to its overall aroma through comparing sensory evaluation results between the incomplete model and complete

model (He et al., 2020). In this test, it is mainly divided into the following four main steps: firstly, accurately quantify the potential volatile substances in the food; secondly, establish a mixing model according to the original concentration; thirdly, remove the volatile substances one by one in a certain order; finally sensory evaluation for omission tests (Engel et al., 2002).

Generally, aroma compounds with OAV ≥ 1 are selected to establish aroma models, and then volatile compounds with lower OAV values would be omitted one by one to obtain a series of incomplete aroma models, and then the effect of removing these volatile compounds on aroma profile would be evaluated (Song & Liu, 2018). The omission test could not only evaluate the contribution of a certain volatile substance to overall aroma, but also further narrow the range of potential volatile substances and reorganize the aroma model with fewer substances. The omission test has become the most popular approach applied for identification or characterization of the key aroma compounds of foods. For obtaining the key ester compounds of apple juice, 19 omission models were established (Y. Niu et al., 2019). Based on the quantitative and OAV results of sample, all of the ester compounds were mixed and added into apple juice model solution to prepare the complete aromatic reconstitution. Each of the omission model was compared with complete aromatic reconstitution by triangular tests. All the tested samples were determined by panel and arranged in a random code (three repetitions). Aroma recombination, addition, and omission experiments of the selected 6 aroma compounds in taste-reconstituted apple juice showed that each compound had an individual aroma profile. Comparison of the overall aroma between this recombination mixture and apple juice showed high similarity, suggesting that the key aroma compounds had been identified successfully.

1.5.6 Sensory evaluation

Sensory analysis is a combination of sensory assessors' visual, olfactory, taste and other sensory organs to evaluate the sensory attributes of food, and combines physiological, psychological, chemical and statistical analysis to evaluate consumers' preference (Jing Zhu & Lv, 2009). In the volatile aroma research, sensory analysis refers to people’s perception of the volatile substances in food through olfactory.

Sensory evaluation methods mainly include difference test method, descriptive analysis method, and consumer test (Liu et al., 2016). The descriptive analysis

method is the most widely used in food sensory analysis. It can accurately analyze the differences between the sensory characteristics of different samples, and obtain consumers' detailed perception of sample attributes, thereby improving the quality of samples (Liu et al., 2016). Quantitative descriptive analysis (QDA) is one of the classic traditional descriptive methods, formed in the Stanford Research Institute in the early 1970s. This method requires multiple (8-12) sensory assessors. Descriptors, definitions, references, etc. describing the differences in the samples were determined through a consensus discussion, and samples were evaluated using linear or non-linear scales (Stone & Sidel, 2004). QDA descriptors are the result of discussions and decisions made by members of the evaluation team, and are made by the evaluators themselves. Sensory evaluation is also used to compare the sensory differences between different recombination models and original samples in the process of omission tests and recombination experiments. Zhang et al., (2021) compared the sensory analysis radar chart of different aroma recombination models and the original clear red raspberry juice, found the grassy, floral and fruity notes had the greater contribution to overall aroma. However, sensory evaluation is a very subjective analysis method, which is affected by age, gender, region, emotion, physical condition, environment and culture (Murray et al., 2001). Through the combination of sensory perception and objective instrumental analysis, it could evaluate aroma characteristics more scientifically and effectively and better explain the relationship between aroma components and sensory experience.

1.5.7 GC-MS/MS

Similarly, tandem mass spectrometry (MS-MS) is also a tandem mass spectrometry technique. Compared with MS, MS-MS has two quadrupoles. The (collision cell) reaction cell is added between the two quadrupoles. The ions screened by the first quadrupole enter the reaction cell for reaction and then pass through the second quadrupole. Two quadrupoles are screened once (Dass, 2007). The advantage is that fragments with the same m/z could be further screened through the reaction, with higher selectivity. It solves the problem of MS, which is low resolution and interference with complex matrix samples that can easily cause false positive results. The MS-MS method has been successfully applied to the aroma detection of fruits, vegetables and their products to determine hundreds of compounds. This method has the characteristics of high sensitivity and strong anti-interference ability, and is currently a more advanced method for aroma analysis

(Rivellino et al., 2013).

1.6. Research progress of red jujube aroma

1.6.1 Aroma of red jujube

At present, the research of red jujube aroma mainly focuses on the free volatiles, including aroma characterization of jujube as affected by regions, varieties and maturities. Wong et al., (1996) detected 78 of aroma compounds by using water vapor extraction combined with GC-MS. Liu et al., (2015a) and Zhang et al., (2018) used the HS-SPME combined with GC-MS analysed different cultivars of red jujube. The results showed that the main aroma components of jujube were acids, aldehydes, ketones, esters, alcohols and heterocyclic compounds. These aroma compounds were also reported in the studies of Deng et al., (2013). Qiao et al., (2022) investigated the volatile compounds of red jujube in different cultivars by using GC-IMS and E-nose. The results showed acetoin, (*E*)-2-hexanol, hexanal, acetic acid, and ethyl acetate played an important role in the classification results. “Huizao” had the most abundant specific volatile compounds and had higher intensity in jujube ID, floral, sweet, and fruity attributes. Moreover, the degree of maturity also had a significant influence on jujube aroma. In contrast to fresh jujube, red jujube is typically harvested during their dried mature stage. Throughout the drying process, the jujube fruit would undergo softening, develop wrinkles on the surface, and experience a reduction in volume until it naturally detaches. Notably, the characteristic aroma of red jujubes would continually develop during the dried mature stage, leading to significant divergence from the aroma of fresh jujubes (Song et al., 2019). While aldehydes and acid compounds dominated the aroma of fresh jujubes, esters and acid compounds dominated the aroma in red jujubes (Huang et al., 2021; Liu et al., 2015). Despite documentation of the aroma profile of red jujubes, the precise identification of the key aroma-active compounds in red jujubes remained elusive. Zhu & Xiao, (2018a) characterized the major odour-active compounds of three cultivars red jujubes by GC-O and OAV, including “Jinsixiaozao”, “Youzao” and “Yuzao”. And hexanal, (*E*)-oct-2-enal, *β*-damascenone, ethyl hexanoate, 3-mercaptohexyl acetate, and 2,5-dimethylpyrazine were identified as key odour-active compounds of “Jinsixiaozao”. Besides, the sensory evaluation showed the notes of sweet and jujube ID were the strongest sensory characteristics of these red jujube.

1.6.2 Aroma of red jujube processed products

At present, 90% of red jujube on the market are consumed in the form of dried red jujube. Drying can prolong the storage of red jujube and improve the flavour of red jujube. The commonly used drying methods mainly include hot air drying, heat pump drying, microwave drying, medium- and short-wave infrared radiation, vacuum drying, freeze drying and instant controlled pressure drop drying (Chen et al., 2014; Song et al., 2020). Almost studies investigated the effect of different drying methods on the red jujube aroma. In the study of Song et al. (2020), they found there were more than 90% of the aroma compounds in dried red jujube were acids. And the freeze-dried red jujube had the highest content of aroma among of these drying methods, while the red jujube had the most diverse aroma compounds after instant controlled pressure drop drying. In the study of Bi et al., (2011) the results showed that the aroma components of dried red jujube were mainly acids, esters, aldehydes and ketones, and their types and contents were significantly higher than those of fresh jujube.

In addition to drying, fermentation is also one of the ways in which red jujube are processed. Sun et al., (2019b) detected the content of volatile compounds in jujube fruits during blacking process. The results showed the total acid content was mild with 8.82 g/kg and increased to 23.45 g/kg by 177.21% with thermal processing for 96 hours. 5-HMF was keep growing to 3.52 g/kg. The volatile component had great change in black jujube fruits compared to untreated jujube. Peng et al., (2011) investigated the aroma content of higher alcohols in jujube wine produced in different wineries and in different aging years, and showed that both the raw material of jujube and the aging time affect the aroma of jujube wine. Zhang et al., (2004) used “Huizao” as raw material and found that “Huizao” wine contained 52 of aroma compounds, mainly alcohols, esters and acids, and found that ultra-high-pressure treatment also affected the aroma of jujube wine.

2. Conclusion and future trends

A systematic introduction to red jujube and its products, the aroma formation in red jujube, the factors affecting aroma of red jujube, and the extraction and analysis of volatile compounds. In addition, the research progress in aroma of red jujube and its products also were discussed. Aroma as an important quality for red jujube and its products. Currently, there are no reports on the comprehensive aroma of red jujube, and few reports to identify and track the key aroma compounds changes after

processing of red jujube. Therefore, the comprehensive aroma and key aroma compounds in red jujube will be studied through HS-SPME combined GC-MS/O and molecular sensory technique. In addition, the key aroma and aroma changes of pilot freeze dried red jujube will also be investigated. Finally, the formation pathway of aroma active compounds in freeze-dried and other typical processed red jujube will be speculated.

3

Chapter III. Comprehensive investigation on free and glycosidically bound volatile compounds in *Ziziphus jujuba* cv. Huizao

Generally, aroma consists of free and glycosidically bound volatile compounds. At present, the research on red jujube aroma mainly focused on free volatiles, including aroma characterization of red jujube as affected by regions, varieties and processing. However, few research has been done on the identification of glycosidically bound volatile compounds and key active aroma. Therefore, this chapter taken the most commonly used processed red jujube, "Huizao", as the research object, carried out a comprehensive study on its free and bound volatile compounds, and identified its key aroma components.

Gou, M., Chen, Q., Qiao, Y., Li, J., Long, J., Wu, X., Zhang, J., Fauconnier, M.L-, Jin, X., Lyu, J., & Bi, J. (2022). Comprehensive investigation on free and glycosidically bound volatile compounds in *Ziziphus jujube* cv. Huizao. Journal of Food Composition and Analysis, 112(March), 104665. https://doi.org/10.1016/j.jfca.2022.104665

Abstract: Both free and glycosidically bound volatile compounds of Ziziphus jujube cv. Huizao were comprehensively investigated in this study. There were 43 and 17 volatile compounds identified in free and bound fraction of "Huizao", respectively. Green, sweet, floral, fruity, cream, sour/rancid and nut notes could describe the aroma profiles through quantitative descriptive analysis. In terms of free volatiles, 22 compounds with odour activity values ≥ 1 and 21 compounds with detection frequency ≥ 2. 3-Hydroxy-2-butanone, butane-2,3-dione, methyl decanoate, ethyl decanoate, methyl dodecanoate, 5-propyloxolan-2-one, 5-butyloxolan-2-one, 2-ethyl-3,5-dimethyl-pyrazine, (*E*)-but-2-enoic acid, hexanoic acid, hexanal, 6-methyl-5-hepten-2-one, 3-oxobutan-2-yl acetate, 5-ethyloxolan-2-one, and 3-methyl-butanoic acid were identified as key aroma-active compounds in "Huizao" via recombination and omission tests. Moreover, key aroma-active compounds of 3-hydroxy-2-butanone, 5-ethyloxolan-2-one, (*E*)-but-2-enoic acid, hexanoic acid and 3-methyl-butanoic acid were also detected in bound fraction, aroma intensity of "Huizao" could be effectively enhanced by enzymatic hydrolysis.

Keywords: red jujube, odour active values, detection frequency analysis, aroma recombination, glycosides

1. Introduction

Jujube (*Ziziphus jujuba* Mill.) belongs to the family Rhamnaceae, which originated in the middle and lower reaches of the Yellow River in China. The cultivation and utilization of jujube in China has a long history of more than 4000 years (Song et al., 2019). Red jujube could be used not only as a fruit, but also as a Chinese folk medicine. The benefits of red jujube is due to its various active ingredients such as polysaccharides, phenols, saponins, cyclic nucleotides, alkaloids, triterpenes, sterols, essential oils, etc. (Chen et al., 2018; Choi et al., 2012). In addition to high nutritional value, red jujube has a unique flavour. Aroma is an important index to evaluate the flavour quality of jujube. Pleasant aroma is one of the important factors to attract consumers and enhance market competitiveness, which directly affects its commodity value.

Generally, aroma consists of free and glycosidically bound volatile compounds. In recent years, the research on jujube aroma mainly focused on free volatiles, including aroma characterization of jujube as affected by regions, varieties and maturities. Qiao et al. (2021) found the obviously differences in volatile compounds of winter jujubes from different regions. As well as varieties showed significant effect on the volatile components, significant differences were observed in contents of alcohols, acids, ketones and esters compounds from ten different varieties of fresh Chinese jujubes. But hexanoic acid, hexanal, (*E*)-hex-2-enal, (*Z*)-hept-2-enal, benzaldehyde and (*E*)-non-2-enal were the main aroma components with contributions that over 70% (Chen et al., 2018). Similar results were also found in Wang et al. (2019) who studied 15 fresh Chinese jujube cultivars aroma compounds. Besides, maturity also has a considerable influence on jujube aroma. Jujube fruit at half-red maturity stage showed better overall flavour quality during the fresh ripening stage (Song et al., 2019). Unlike fresh jujube, red jujubes were usually harvested in the dried mature period. In the process of dried ripening, the jujube fruit would be softened, wrinkles would appear on the surface, and the volume would be shirked until it falls off naturally. More importantly, the characteristic aroma of red jujube would be formed continuously in the dried mature period which presented significant difference with that of fresh jujube. The aroma of jujube between dried mature period and fresh mature period were quite different. Aldehydes and acids compounds were main volatiles in fresh jujube, while, the esters and acids

compounds were dominant volatiles in dried jujube (Huang et al., 2021; Liu et al., 2015). Although aroma profile of red jujube was reported, while, the key aroma-active compounds of red jujube were still unclear. With the development of molecular sensory technology, more and more key aromas of fruits were identified, which is proposed by Professor Steinhaus and Schieberle in 2007, mainly including GC-MS and application of gas chromatography−olfactometry (GC-O), combined with odour active value (OAV), omission test and aroma reconstitution experiment (Steinhaus & Schieberle, 2007). Zhu & Xiao (2018) determined the odour-active compounds of three cultivars red jujubes by GC-O and OAV, including "Jinsixiaozao", "Youzao" and "Yuzao". Hexanal, (*E*)-oct-2-enal, *β*-damascenone, ethyl hexanoate, 3-mercaptohexyl acetate, and 2,5-dimethylpyrazine were identified as key odour-active compounds of "Jinsixiaozao". But, "Huizao", as one of the most popular cultivars of red jujube, little information has been reported on its key aroma yet.

As a potentially important source of aroma, glycosidically bound volatile compounds have not been noticed on red jujube aroma. Whereas, some bound aroma have been studied in some fruits, such as kiwifruit, citrus fruits, cherry, grape and strawberry, etc. (Garcia et al., 2012; Ren et al., 2015a; Ubeda et al., 2012; Wen et al., 2014a; Wu et al., 2020). Generally, glycosidic precursors could generate free volatiles after hydrolysis, such as monoterpenes, C13-norisoprenoids, benzenic compounds, hydroxy esters, and fatty alcohols (Ren et al., 2015a), thus increasing and changing the flavour. Enzymatic hydrolysis is a widely used method to release glycosides. And the glycosidic bond between the glucopyranosyl unit and the aglycone moiety could be hydrolyzed effectively by AR2000 (Chen et al., 2020). Thus, it is of great significance to investigate the bound aroma of red jujube through AR2000 for its development and utilization.

Therefore, the overall aroma profile of "Huizao" was comprehensively evaluated from both free and glycosidically bound volatile compounds. Moreover, the key aroma-active compounds of "Huizao" were further identified based on molecular sensory science technology, including GC-MS, GC-O, aroma recombination and omission test. This study will be helpful to comprehensively understand the aroma characteristic of "Huizao" and provide guidelines for its practical processing.

2. Materials and methods

2.1. Materials and chemicals

Red jujubes (*Ziziphus jujuba* cv. Huizao) were harvested in the dried mature period from local orchard in Akesu, Xinjiang Province, China during the November 2020. Red jujubes performed purplish-red color without any physical damage were selected, then collected and transported to Beijing within 2 days. All red jujube samples were stored at 4 ^{o}C controlled atmosphere storage room until use. The water content of "Huizao" was 25.57%, the pH was 5.5, and the solid soluble content was 69.0%.

Oct-1-en-3-ol, hexanal, (*E*)-oct-2-enal, decanal, benzaldehyde, butane-2,3-dione, octan-3-one, 3-hydroxy-2-butanone, oct-1-en-3-one, 6-methyl-5-hepten-2-one, 3-oxobutan-2-yl acetate, 3-methyl-butanoic acid, (*E*)-but-2-enoic acid, hexanoic acid, heptanoic acid, (*E*)-2-hexenoic acid, methyl hexanoate, methyl heptanoate, methyl decanoate, ethyl decanoate, methyl benzoate, methyl dodecanoate, 5-ethyloxolan-2-one, 5-propyloxolan-2-one, 5-butyloxolan-2-one, 2-ethyl-6-methyl-pyrazine, D-limonene, styrene, 2-ethyl-3,5-dimethyl-pyrazine, 1-methyl-4-propan-2-ylbenzene, and naphthalene were purchased from Yuanye Bio-Technology (Shanghai Yuanye Bio-Technology Co., Ltd) or Macklin (Shanghai Macklin Biochemical Co., Ltd). All of the chemical standards used above with purity ≥ 99%.

2.2. Extraction of free volatiles from jujube by HS-SPME

A 65 μm polydimethylsiloxane-divinnylbenzene (PDMS/DVB) fiber and PAL RTC automatic sampling device (Guangzhou Ingenious Laboratory Technology Co., Ltd) were applied in this study. The extraction as described by Song et al. (2020). Briefly, the kernel of jujubes was removed and the remaining part was cut into 5 mm slices; then crushed using a Joyoung pulverizer (JYL-CO20, Joyoung Co., Ltd., Shandong, China). Then 2 g of jujube sample were added to a 20 mL headspace bottle and supplemented with 2 μL internal standard (2-cyclohexen-1-one, 1 mg/mL) (Qiao, Chen, et al., 2022). The sample was incubated at 50 °C for 40 min to reach equilibrium. Afterward, the fiber was inserted into the sample vial and exposed to the headspace environment with continuous heating (50 °C) and agitation (250 rpm) for 30 min. After extraction, the SPME fiber was withdrawn and directly insert into the GC injector for desorbing at 250 °C for 3 min.

2.3. Extraction of bound volatiles from jujube by Amberlite

XAD-2 resin

The extraction of bound volatiles from red jujubes were referred to Garcia et al. (2012) and Yang et al. (2019) with small modification. The 150 g red jujube slices and Milli-Q deionised water (450 mL) were blended together by the Joyoung pulverizer. The mixtures were centrifuged for 20 min (1200 r/min, 4℃) and obtained the supernatant.

The 200 mL supernatant jujube juice was adsorbed through 50 g Amberlite XAD-2 resin. Then washing water-soluble sugars, acids and other polar compounds in resin with 300 mL deionized water, followed by remove the free volatile compounds with 300 mL diethyl ether-pentane (1:1, V/V). Next, the retained bound compounds were eluted with 300 mL of methanol. The methanol eluent was collected and concentrated on a rotary evaporator under vacuum (water bath temperature 35 ℃) to dryness, and then, reconstitute with 40 mL of 0.05 mol/L citric acid/disodium hydrogen phosphate buffer (pH 5.0). Then 150 mL diethyl ether-pentane solution (1:1 v/v) was used to extract three times to remove the possibly free volatile compounds, and the aqueous phase is ready for use.

Accurate 10 mg β-glucosidase (9.46 U/mg) (Ren et al., 2015a) was added into a headspace bottle containing the obtained aqueous phase solution, and sealed with a polytetrafluoroethylene septum. Then the sample hydrolyzed at 40 ℃ for 48 h. Thereafter, 30 mL of ether/pentane solution (1:1 v/v) was used to extract the enzymatic hydrolysis in three times. The extract was dried over anhydrous sodium sulfate, then was concentrated to 1 mL with N_2, 2 μL of internal standard (2-cyclohexen-1-one, 1 mg/mL) was further added, which could be used for GC-MS analysis. Meanwhile, the sample without enzymatic hydrolysis was used as a control.

2.4. GC-MS analysis

A 7890B gas chromatograph with a 5977 mass selective detector (MSD, Agilent Technologies, Inc., Santa Clara, CA, U.S.A.) was used. The volatile compounds of jujube sample were analyzed on DB-WAX columns (60 m×0.25 mm×0.25 μm; Agilent Technologies). The oven temperature was programmed to rise from 40 ℃ (held for 3 min) to 90 ℃ at 7 ℃/ min and subsequently to 120 ℃ at 4 ℃/min, then it was increased to 170 ℃ at 5 ℃/min, thereafter, it was increased to 200 ℃ at 4 ℃/min and maintained for 8 min. The carrier gas was helium at the flow rate of 1 mL/min with splitless mode. The MS fragmentation was performed by electronic

impact (EI) mode. The acquisition was full-scan mode with the range of 35-550 m/z. Internal standard method was used for aroma quantitative analysis (2 μL 2-cyclohexen-1-one, 1 mg/mL). The content of each volatile compound was calculated based on the GC peak areas related to that of internal standard.

The quantitative identification of 31 odour active compounds of jujubes was determined by calibration curves constructed. The conditions were as same as described for the GC-MS analysis above mentioned. The standard curves for different compounds ($R^2 > 0.99$) were established. The calculation of each identified compound was according to the standard curve and results were shown in Table 3-1.

2.5. GC-O

A sniffing port (Sniffer 9000, Brechbühler, Schlieren, Switzerland) coupled to the GC-MS instrument was used to discriminate the aroma-active compounds in the HS-SPME isolates. At the end of the capillary column, the effluent was divided equally by volume between the sniffing port and the MS detector. The transfer line to the GC-O sniffing port was maintained at 280 °C (Liu et al., 2018). GC-O was performed by three experienced panelists. The odour characteristics and detection frequency (DF) of the aroma compounds would be recorded by panelists. The compounds could be considered as having aroma potential activity with DF ≥ 2 (reported by at least two assessors).

2.6. Odour activity value (OAV)

OAV was calculated according to OAV = C/OT, where C was the concentration of the compound and OT was its orthonasal detection odour threshold. The threshold values of volatile compounds in water referred to the literature.

2.7. Aroma recombination and omission experiments

To identify the key aroma-active compounds based on detection frequency analysis (DFA) and OAV results, triangle tests were carried out. Different aroma mixture models were prepared by omitting one or a group of selected compounds from the complete recombinant aroma model. Each omission sample was evaluated against complete recombination model prepared by mixing the standard aroma compounds at the concentrations in raw jujube (recombination model 1). If there is a significant difference, it means that the missing aroma component is the key aroma-active compound of “Huizao”. On this basis, the key aroma-active compounds were selected for the recombination test (recombination model 2). Sensory evaluation

analyses the similarity of the odour between the recombinant model 2 and raw jujube, and determines the aroma characteristics of "Huizao". In the end, 10 sensory evaluators (23-27 years old, 4 males and 6 females) who were experienced and engaged in food flavour research was selected for sensory evaluation. The analysis was as follows: significant ($\alpha \leq 0.05$), if 7 people answered correctly; highly significant ($\alpha \leq 0.01$), if 8 people answered correctly; very highly significant ($\alpha \leq 0.001$), if 9 or 10 people answered correctly. The sensory panel of the omission experiments was the same as that of aroma profile evaluation (Sun et al., 2021).

2.8. Sensory evaluation

The quantitative descriptive analysis (QDA) was often applied to investigate the differences between raw jujube samples and models samples. In this study, 3-hydroxy-2-butanone (cream), 5-butyloxolan-2-one (floral), hexanal (green), methyl dodecanoate (fruity), 2-ethyl-6-methyl-pyrazine (nut), acetic acid (sour), 3-methyl-butanoic acid (rancid) and (*E*)-but-2-enoic acid (sweet) were the reference compounds of aroma descriptors which were dissolved in water at a concentration of 100 times of their respective odour threshold (Zhang et al., 2021). The synthetic aroma solution samples and the original jujube were coded with three-digit numbers randomly. QDA was conducted in triplicate by a trained panel (23-27 years old, 4 males and 6 females, above mentioned). Scores for each sample in 0.5 increments, from 0.0 to 3.0 on the basis of 7 point scales (0, none; 1.5, moderate; and 3, very strong).

2.9. Statistical Analysis

Duncan's multiple tests were performed with Software of SPSS version 20.0 (SPSS Inc., Chicago, IL). The data were illustrated using Origin 2018 (OriginLab Corporation, Northampton, MA). The venn graph was performed by http://jvenn.toulouse.inra.fr/app/example.html. Contents of different components were presented as the mean ± SD (standard deviation).

3. Results and discussion

3.1. Free aroma profile of "Huizao"

A total of 43 free volatile compounds were detected in "Huizao", including aldehydes (6), alcohols (2), acids (7), esters (9), ketone (6), lactones (4), pyrazines (4), olefins (3), 1-methyl-4-propan-2-ylbenzene and naphthalene **(Table 3-1)**, of

which 33 components had been reported in previous studies on fresh jujube, red jujube and dried jujube (Chen et al., 2018; Hernández et al., 2016; Huang et al., 2021; Liu et al., 2015; Wang et al., 2018, 2019; Zhu & Xiao, 2018). While, 10 aroma compounds were identified for the first time in "Huizao", including 3-oxobutan-2-yl acetate, hexyl acetate, oxolan-2-one, 5-propyloxolan-2-one, 2-ethyl-6-methyl-pyrazine, 2-ethyl-3,5-dimethyl-pyrazine, tetramethyl-pyrazine, limonene, styrene and α-farnesene. The difference of volatile compounds between "Huizao" and previous report might be caused by origin and variety.

Table 3-1 Free volatile compounds identified in "Huizao" jujube and aroma-active compounds obtained from DFA and OAV.

No	Compounds	LRI[a]	LRI[b]	Identification method[c]	Concentration (μg/kg)[d,e]	OAV[f]	DF[g]	Odour description[h]
Alcohol								
A1	Oct-1-en-3-ol*	1450	1448	MS/RI/O/Std	157.699±4.761	105	6	earth, fat, floral, mold, mushroom
A2	Butane-2,3-diol	1543	1553	MS/RI/Std	27.281±9.740	<1	-	
Aldehyde								
B1	Hexanal*	1083	1078	MS/RI/Std	49.083±0.002	10	-	
B2	(*E*)-Hex-2-enal	1216	1228	MS/RI/Std	9.117±1.035	<1	-	
B3	(E)-Oct-2-enal*	1429	1410	MS/RI/O/Std	45.366±0.229	15	2	green, nut, fat
B4	Furan-2-carbaldehyde	1460	1449	MS/RI/Std	46.624±0.717	<1	-	
B5	Decanal*	1480	1498	MS/RI/Std	16.799±0.032	6	-	
B6	Benzaldehyde*	1520	1508	MS/RI/O/Std	242.605±11.400	<1	4	bitter almond, burnt sugar, cherry, malt
Ketone								
C1	Butane-2,3-dione*	997	979	MS/RI/O/Std	76.187±10.682	76	6	butter, caramel, cheese, cream, fruit
C2	Octan-3-one*	1253	1240	MS/RI/Std	22.972±0.492	1	-	
C3	3-Hydroxybutan-2-one*	1284	1286	MS/RI/O/Std	741.780± 11.861	53	4	butter, cream, green pepper, rancid, sweat
C4	Oct-1-en-3-one*	1290	1296	MS/RI/O/Std	11.765±0.133	3922	4	earth, green, metal, mushroom
C5	6-Methyl-5-hepten-2-one*	1338	1342	MS/RI/O/Std	46.004±0.118	1	4	citrus, mushroom, pepper, rubber, strawberry
C6	3-Hydroxybutan-2-one acetate*	1378	/	MS/RI/O/Std	9.600±0.038	-	6	cream, sweet, fat
Acids								
D1	Acetic acid	1449	1429	MS/RI/Std	550.443 ±183.011	<1	-	
D2	3-Methyl-butanoic acid*	1666	1680	MS/RI/O/Std	81.491±3.939	<1	2	cheese, fecal, putrid fruit, rancid, sweat
D3	(*E*)-But-2-enoic acid*	1745	1750	MS/RI/O/Std	88.874±2.328	-	2	sweet, cream, butter, fat
D4	Hexanoic acid*	1846	1849	MS/RI/O/Std	1583.531±49.854	2	3	acid, cheese, goat, pungent, rancid
D5	Heptanoic acid*	1950	1943	MS/RI/O/Std	154.352±13.243	<1	2	apricot, floral, rancid, sour, sweat
D6	(*E*)-hex-2-enoic acid*	1967	1994	MS/RI/O/Std	23.084±0.257	-	2	fat, must
D7	Octanoic acid	2060	2086	MS/RI/StdI	118.744±5.732	<1	-	

Esters								
E1	Methyl acetate	828	810	MS/RI	471.541±90.376	<1	-	
E2	Methyl hexanoate*	1184	1177	MS/RI/Std	1442.026±32.094	21	-	
E3	Hexyl acetate	1271	1265	MS/RI/O/Std	14.746±2.607	<1	-	
E4	Methyl heptanoate*	1287	1302	MS/RI/Std	206.720±5.790	52	-	
E5	Methyl decanoate*	1593	1636	MS/RI/Std	1164.020±52.647	271	-	
E6	Ethyl decanoate*	1605	1633	MS/RI/O/Std	273.481±15.010	<1	4	brandy, burnt, grape, nut, pear
E7	Methyl benzoate*	1612	1631	MS/RI/Std	272.989±14.898	4	-	
E8	Methyl dodecanoate*	1804	1834	MS/RI/O/Std	1204.607±77.962	803	6	coconut, fruit, sweet
E9	Methyl hexadecanoate	2194	2243	MS/RI/Std	79.909±38.922	<1	-	
Lactones								
F1	Oxolan-2-one	1632	1602	MS/RI	149.547±10.932	<1	-	
F2	5-Ethyloxolan-2-one*	1694	1736	MS/RI/O/Std	72.090±0.345	<1	5	coconut, coumarin, onion, sweet, warm
F3	5-Propyloxolan-2-one*	1787	1796	MS/RI/O/Std	30.354±0.210	<1	2	caramel, fat, nut, peach, sweet
F4	5-Butyloxolan-2-one*	1910	1936	MS/RI/O/Std	31.849±0.334	5	4	coconut, fruit
Pyrazines								
G1	2,6-dimethyl-Pyrazine	1328	1319	MS/RI/Std	5.002±0.002	<1	-	
G2	2-ethyl-6-methyl-Pyrazine*	1386	1363	MS/RI/O/Std	54.455±3.182	1	2	green, nut, roasted
G3	2-ethyl-3,5-dimethyl-Pyrazine*	1455	1464	MS/RI/O/Std	115.493±11.501	2887	5	earth, must, nut, potato, roast
G4	Tetramethyl-pyrazine	1470	1457	MS/RI/Std	47.528±0.269	<1	-	
Olefins								
H1	Limonene*	1200	1189	MS/RI/O/Std	174.833±0.641	5	4	Citrus, orange
H2	Styrene*	1261	1254	MS/RI/Std	88.559±0.283	1	-	
H3	α-Farnesene	1746	1754	MS/RI/Std	11.266±0.657	-	-	
Others								
I1	1-Methyl-4-propan-2-ylbenzene*	1270	/	MS/RI/Std	10.721±0.553	2	-	
I2	Naphthalene*	1746	1707	MS/RI/Std	87.725±0.493	15	-	

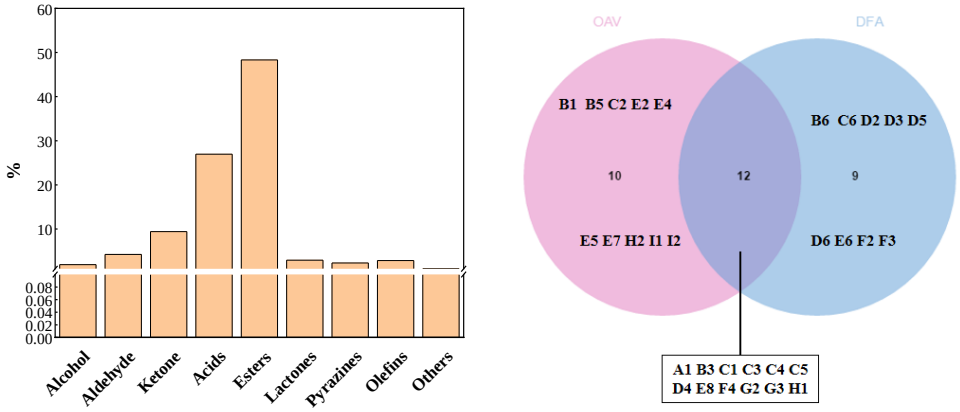
[a] Linear retention index on DB-Wax columns were determined by *n*-alkanes.

[b] Linear retention index on DB-WAX column from the literature. And "/" indicated the compound lack of reliable linear retention index value in literature.

[c] MS, identified by MS spectra; LRI, linear retention indices; O, identified by comparison of their odour description with the authentic compounds *via* GC–O; S, identified by comparison to standards.

[d] Concentration was calculated via an internal standard method.
[e] Standard deviation.
[f] OAV was equal to the odour concentration divided by the threshold in water. The threshold was obtained from information available in the website (https://www.vcf-online.nl) and L. J. van Gemert.And "-" indicated the compound lack of reliable odour threshold data.
[g] Sum of times detected by three assessors during DFA.
[h] Odour description perceived by the judges during DFA.
* Volatiles (with DFs ≥ 2 and OAVs ≥ 1) identified as the major aroma-active compounds.

Among all the volatiles in "Huizao", esters contributed the highest quantitative volatile portion (48.32%), **(Figure 3-1(a))**. While, acids and aldehydes were the main aroma compounds in fresh jujubes (Chen et al., 2018; Liu et al., 2021; Song et al., 2019, 2020; Wang et al., 2019). That could be due to the ester compounds would be accumulated and formed continuously during the dry ripening of jujube. Esters are the most important compounds in the aroma components of mature fruits. These small molecular esters might be the secondary metabolites of some fatty acid methyl esters (Padilla-Jiménez et al., 2021; J. Yan et al., 2018). Most of them have fruity note, which play an important role in the formation of the key aroma of "Huizao". In addition, 4 lactones were detected in "Huizao". The acids (26.96%) and ketone (9.42%) are also important compounds that contribute to sour, sweet and cream notes. As well as aldehyde (4.25%), lactones (2.95%) and pyrazines (2.30%) also had some contributions to the "Huizao" overall aroma profiles, but they showed lower contents in jujubes. Other components showed little contribution to the flavour of "Huizao".



(a) (b)

Figure 3-1 The aroma-active compounds in "Huizao". (a) Content percentages of the major odour classes in "Huizao". (b) Distribution of aroma-active compounds identified in "Huizao" by odour active values (OAV) and detection frequency analysis (DFA) (numbering refers to **Table 3-1**).

3.2. Identification of key aroma-active compounds in "Huizao"

3.2.1. Identification of aroma-active compounds by OAV

The OAV could provide a reasonable evaluation of aroma potency based on the

equilibrium between the food matrix and the air (Wentao Zhang et al., 2019b). **Table 3-1** presented the OAVs of 39 volatile compounds with their available odour threshold data found in the literature (Gemert, 2011) or website (https://www.vcf-online.nl/VcfHome.cfm). Twenty-two compounds with OAV ≥ 1 were identified as aroma-active compounds, of which oct-1-en-3-one had the highest OAV of 3922 with earth, green, metal and mushroom-like notes, followed by 2-ethyl-3,5-dimethyl-pyrazine with OAV of 2887 with earth, must, nut, potato, and roast-like notes. Otherwise, methyl dodecanoate (OAV=803), methyl decanoate (OAV=271), oct-1-en-3-ol (OAV=105), butane-2,3-dione (OAV=76) and 3-hydroxy-2-butanone (OAV=53) and methyl heptanoate (OAV=52) also had the higher OAVs. Furthermore, these volatile compounds were also detected in other cultivars, such as, "Jinsixiaozao", "Youzao", "Yuzao", "Tangzao", "Muzao", "Lizao", and "Qingrunhongzao" (Chen et al., 2018; Hernández et al., 2016; Song et al., 2019; Wang et al., 2018, 2019). Although, (*E*)-2-hexenal (OAV < 1) and benzaldehyde (OAV < 1) had the lower OAVs, they were also considered the major aroma-active in jujube. That might be due to benzaldehyde perform an additive or synergistic function at sub-threshold concentrations resulted the overall aroma of red jujube perceived more easily (Jiancai Zhu & Xiao, 2018a). In contrast, though acids were dominant in quantity, they had the lower OAVs, due to their relatively high odour threshold. For instance, hexanoic acid was the most abundant compound (1583.531 μg/kg) but with low OAV value (only 2). Due to the OAV calculated based on concentration and threshold of aroma compound, the GC-O was further applied for identification of aroma-active compounds.

3.2.2. Identification of aroma-active compounds by DFA

Some aroma components with important sensory contributions are difficult to be detected by instruments due to their low content. In order to solve this problem, GC-O technology came into being. DFA method is the simplest GC-O method and the easiest to operate (Gou et al., 2021). There are 21 compounds were identified by DFA which were categorized into 7 groups according to their odour characteristics. The first group featured fruit-like odourants (B6, C1, C5, E6, E8, F4 and H1 in **Table 3-1**) described as cherry, strawberry, peach, lemon, orange and coconut. The second group mainly covered the floral-like odourants (A1 and D5 in **Table 3-1**). The third group consisted of the sweet-like odourants (C3, C6, D3, F2 and F3 in **Table 3-1**). The fourth group contributes the green and grass aroma (B3, and C4 in

Table 3-1). The fifth group contributes the cream and butter odour (C1, C3 and C6 in **Table 3-1**). Compounds (D2, D4 and D6 in **Table 3-1**) and compounds (G2 and G3) comprised the last two groups described as off-flavour, with descriptors of rosin, sour, sweat, spice, and roast, baking and nut. Oct-1-en-3-ol with fat, mushroom and floral notes, butane-2,3-dione with cream and fruit notes, 3-oxobutan-2-yl acetate with cream, sweet and fat notes, and methyl dodecanoate with coconut, fruit and sweet notes were recognized by all the panelists, revealing that they contributed more actively than other compounds to the overall aroma of "Huizao". However, the important contribution of oct-1-en-3-ol, butane-2,3-dione, 3-oxobutan-2-yl acetate and methyl dodecanoate to jujube aroma were underestimated. In addition, 9 compounds, including benzaldehyde (bitter almond), 3-hydroxy-2-butanone (cream), 6-methyl-5-hepten-2-one (citrus), oct-1-en-3-one (green), ethyl decanoate (pear), 5-ethyloxolan-2-one (coconut and sweet), 5-butyloxolan-2-one (coconut and fruit), 2-ethyl-3,5-dimethyl-pyrazine (earth and nut) and limonene (orange) were also recognized as the main contributors in the aroma profile due to their relatively higher DF ($6 > DF \geq 4$). The other 8 compounds ($4 > DF \geq 2$), (*E*)-oct-2-enal (green), hexanoic acid (sour), 3-methyl-butanoic acid (rancid), (*E*)-but-2-enoic acid (sweet), heptanoic acid (rancid and sour), (*E*)-2-hexenoic (fat), 5-propyloxolan-2-one (sweet) and 2-ethyl-6-methyl-pyrazine (nut) were considered as the potential contributors in the aroma profile of "Huizao".

3.2.3 Comparison of DFA and OAV identification

There are 31 aroma-active compounds identified from OAV or DFA (**Figure 3-1(b)**). Twelve components were identified as aroma-active compounds of "Huizao" by both OAV and DFA, namely oct-1-en-3-ol, (*E*)-oct-2-enal, butane-2,3-dione, 3-hydroxy-2-butanone, oct-1-en-3-one, 6-methyl-5-hepten-2-one, hexanoic acid, methyl dodecanoate, 5-butyloxolan-2-one, 2-ethyl-6-methyl-pyrazine, 2-ethyl-3,5-dimethyl-pyrazine and limonene. There were 10 compounds detected only by OAV, while 9 compounds were detected only by DFA. 3-Oxobutan-2-yl acetate was discriminated by all the assessors in DFA, but lack of the odour threshold data, so the OAV value was not available. While, (*E*)-but-2-enoic acid and (*E*)-hex-2-enoic acid were not identified as aroma-active compounds by OAV for the same reason. In addition, there were compounds with high OAV, but not be recognized by DFA, such as methyl decanoate (OAV=271), methyl heptanoate (OAV=52). The difference between the OAV and DFA results might due to their different analysis

principles. The OAV values were calculated based the threshold in water, however, in the jujube matrix, aroma release would be influenced by the interaction between volatiles and food components (Zhang et al., 2019). While, the DFA depends on the human olfaction and the variance would be appeared due to the evaluators' state. Hence, OAV and DFA were synergetic used to identify the aroma active compounds.

3.2.4 Aroma recombination and omission experiments

In order to verify the key aroma compounds of "Huizao", aroma recombination and omission tests were undertaken, and 31 aroma-active compounds (OAV ≥ 1 or DF ≥ 2) were quantified again by calibration curves (**Table 3-2**), the models for the recombination and omission were prepared at original concentrations of these compounds. As shown in **Figure 3-2**, fruity, sweet, floral, roasting, sour and green were selected as the descriptive words according to QDA. Among them, the sweet note received the highest score, followed by fruity, roasting, sour, and green. Also, the aroma profile of the complete recombinant (Model 1, **Figure 3-2**) was similar to that of the original jujube (**Figure 3-2**). This result indicated the success in identification and quantitation of key aroma compounds of "Huizao", because the mixtures of these odourants are very similar to those of the original samples. In a final experiment, through omitting single aroma-active compound or a group of aroma compounds, the contribution of them to the overall aroma of jujube was evaluated. If there is a significant difference between incomplete and complete aroma models, it means that the removed aroma compound is the key aroma-active compound of "Huizao".

Table 3-2 Calibration equations, coefficients of determination (R^2) and concentrations of aroma-active compounds in "Huizao" jujube

No.[a]	Compounds	Calibration equations[b]	R^2	Concentration (μg/kg)[c]
A1	Oct-1-en-3-ol	y=9.708x+0.152	0.999	0.628±0.490
B1	Hexanal	y=1.065x+0.031	0.988	17.074±0.002
B3	(*E*)-Oct-2-enal	y=2.833x+0.044	0.998	0.341±0.081
B5	Decanal	y=1.704x+0.016	0.999	0.469±0.019
B6	Benzaldehyde	y=2.237x+0.064	0.999	80.051±5.095
C1	Butane-2,3-dione	y=0.133x-0.242	0.979	2388.624±80.315
C2	Octan-3-one	y=12.393x+0.010	0.999	1.071±0.040
C3	3-Hydroxybutan-2-one	y = 0.060x +0.142	0.980	9242.388±205.874
C4	Oct-1-en-3-one	y=2.221x+0.012	0.989	0.119±0.060

C5	5-Hepten-2-one, 6-methyl-	y=6.005x+0.041	0.999	0.883±0.020
C6	3-Hydroxybutan-2-one acetate	y=0.002x+0.010	0.966	497.901±189.249
D2	Butanoic acid, 3-methyl-	y=0.034x +0.014	0.955	1952.977±79.187
D3	(E)-But-2-enoic acid	y=0.067x+0.007	0.980	1204.872±35.812
D4	Hexanoic acid	y=0.746x-0.044	0.996	2180.263±66.802
D5	Heptanoic acid	y=1.202x-0.036	0.998	158.665±11.021
D6	(*E*)-hex-2-enoic acid	y=0.007x-0.005	0.968	4040.626±36.733
E2	Methyl hexanoate	y=1.037x+0.035	0.967	135.431±30.940
E4	Methyl heptanoate	y=0.059x-0.009	0.910	367.669±98.642
E5	Methyl decanoate	y=84.091x+0.845	0.999	3.789±0.626
E6	Ethyl decanoate	y=6.976x-0.220	0.996	31.608±0.020
E7	Methyl benzoate	y=1.633x+0.144	0.993	79.121±9.124
E8	Methyl dodecanoate	y=102.655x+0.652	0.998	5.380±0.759
F2	5-Ethyloxolan-2-one	y=0.348x+0.002	0.999	202.042±0.993
F3	5-Propyloxolan-2-one	y=0.143x+0.018	0.999	86.208±1.463
F4	5-Butyloxolan-2-one	y=1.084x+0.001	0.999	28.287±0.308
G2	Pyrazine, 2-ethyl-6-methyl-	y=2.331x+0.015	0.999	0.223±0.004
G4	Pyrazine, 2-ethyl-3,5-dimethyl-	y=1.467x-0.014	0.999	88.004±7.840
H1	Limonene	y=9.267x+0.165	0.995	0.001±0.000
H2	Styrene	y=9.731x+0.078	0.996	0.890±0.029
I1	1-Methyl-4-propan-2-ylbenzene	y=0.025x+0.001	0.993	389.502±22.385
I2	Naphthalene	y=29.367x+0.084	0.998	0.113±0.017

[a]The numbers assigned to the compounds are consistent with those in **Table 3-1**. [b]Variables: y is the peak area relative to that of the internal standard, 2-cyclohexen-1-one, and x is the concentration ($\mu g kg^{-1}$) in the jujube sample relative to that of the internal standard, 2-cyclohexen-1-one. [c]The concentrations are the means of three repeated measurements ±standard deviation (to three significant figures).

As shown in **Table 3-3**, there are 38 omission samples prepared; these samples would be compared to the complete recombination model. All the aldehydes, all the ketones, all the esters, all the acids, all the lactones, and all the pyrazines were key groups of aroma compounds were identified in the omission experiments via triangle tests. A very highly significant difference in aroma was perceived when butane-2,3-dione, 3-hydroxy-2-butanone, methyl decanoate, ethyl decanoate, methyl dodecanoate, 5-propyloxolan-2-one, 5-butyloxolan-2-one, 2-ethyl-3,5-dimethyl-pyrazine, (*E*)-but-2-enoic acid and hexanoic acid were omitted, which indicated these 10 compounds contributed most to the overall aromas of "Huizao" jujube. Besides, the omission of hexanal, 6-methyl-5-hepten-2-one, 3-oxobutan-2-yl acetate,

3-methyl-butanoic acid, 5-ethyloxolan-2-one resulted in highly significant or significant changes in aroma, were also identified as key aroma of "Huizao" jujube. Interestingly, ethyl decanoate identified in the omission experiments on jujube was not calculated to have a high OAV. This might be the OAV calculation was based on the odour threshold values in water, not the real jujube matrix. Similarly, methyl heptanoate and methyl hexanoate were calculated to have high OAVs in jujube; however, they did not play highly significant roles in the omission tests. That might be ascribed to the interactions between aroma compounds or interactions between aroma compounds and the jujube matrix.

Table 3-3 Results of omission experiments performed on aroma reconstitutes of jujube

Number	Compound(s) omitted	N^a	Significance[b]
1	**all alcohol** (Oct-1-en-3-ol)	2	-
2	**all aldehyde**	7	*
2-1	Hexanal	7	*
2-2	Decanal	2	-
2-3	(*E*)-Oct-2-enal	2	-
2-4	Benzaldehyde	6	-
3	**all ketone**	10	***
3-1	Butane-2,3-dione	10	***
3-2	Octan-3-one	5	-
3-3	3-Hydroxybutan-2-one	10	***
3-4	Oct-1-en-3-one	6	-
3-5	6-Methyl-5-hepten-2-one	8	**
3-6	3-Hydroxybutan-2-one acetate	8	**
4	**all acids**	10	***
4-1	3-Methyl-butanoic acid	8	**
4-2	(*E*)-But-2-enoic acid	9	***
4-3	Hexanoic acid	9	***
4-4	Heptanoic acid	4	-
4-5	(*E*)-hex-2-enoic acid	6	-
5	**all esters**	10	***
5-1	Methyl hexanoate	6	-
5-3	Methyl heptanoate	6	-
5-4	Methyl decanoate	9	***
5-5	Ethyl decanoate	9	***
5-6	Methyl benzoate	4	-
5-7	Methyl dodecanoate	10	***
6	**all lactones**	10	***
6-1	5-Ethyloxolan-2-one	8	**
6-2	5-Propyloxolan-2-one	9	***
6-3	5-Butyloxolan-2-one	10	***
7	**all pyrazines**	10	***
7-1	2-ethyl-6-methyl-pyrazine	6	-

7-2	2-ethyl-3,5-dimethyl-pyrazine	10	***
8	**all olefins**	3	-
8-1	Limonene	3	-
8-2	Styrene	2	-
9	1-Methyl-4-propan-2-ylbenzene	5	-
10	Naphthalene	2	-

[a]Number of correct judgments from 10 panelist evaluating the aroma difference by means of a triangle test. [b]Significance: ***, very highly significant ($\alpha \leq 0.001$); **, highly significant ($\alpha \leq 0.01$); and *, significant ($\alpha \leq 0.05$)

Based on the results of omission tests, the aroma recombination model 2 (**Figure 3-2**) was estimated, which is consisted of hexanal, 3-hydroxy-2-butanone, butane-2,3-dione, 6-methyl-5-hepten-2-one, 3-oxobutan-2-yl acetate, methyl decanoate, ethyl decanoate, methyl dodecanoate, 5-ethyloxolan-2-one, 5-propyloxolan-2-one, 5-butyloxolan-2-one, 2-ethyl-3,5-dimethyl-pyrazine, 3-methyl-butanoic acid, (*E*)-but-2-enoic acid and hexanoic acid. Compared the model 2 with raw "Huizao", the aroma profile showed some similarity to the original "Huizao". This result illustrated these 15 compounds could be identified as the key aroma compounds of "Huizao". However, the intensity of floral note in model 2 was rated slightly higher than that in the original jujube and model 1. These differences were due to several reasons, including volatile compounds with sub-threshold concentrations might also contribute to the overall aroma, the information on the complete aroma profile was unavailable at the current technology level, hexanal, oct-1-en-3-ol, and benzaldehyde would reduce the overall threshold value to differing extents (Jiancai Zhu & Xiao, 2018a).

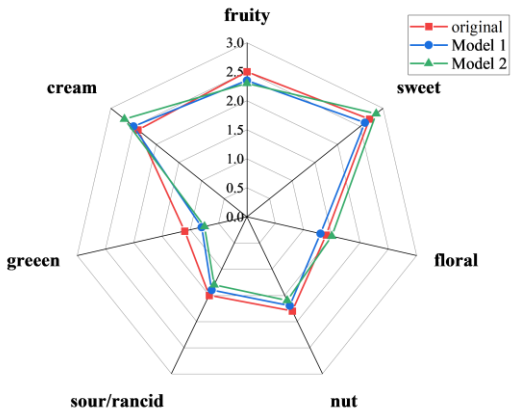


Figure 3-2 Aroma profiles of "Huizao" obtained from original sample, model 1, and model 2.

The results of recombination and omission experiments revealed that 2-ethyl-3,5-dimethyl-pyrazine contribute a positive note of nut and roast flavour to "Huizao". The fruity and floral odours were mainly derived from esters and lactones, the sweet and cream odour were attributed to ketones and esters, the rancid and sour flavour was positively correlated with acids. 3-Hydroxy-2-butanone, butane-2,3-dione, methyl decanoate, ethyl decanoate, methyl dodecanoate, 5-propyloxolan-2-one, 5-butyloxolan-2-one, 2-ethyl-3,5-dimethyl-pyrazine, (*E*)-but-2-enoic acid and hexanoic acid formatted the basic "jujube ID". Hexanal, 6-methyl-5-hepten-2-one, 3-oxobutan-2-yl acetate, 5-ethyloxolan-2-one, and 3-methyl-butanoic acid enriched the "Huizao" aroma.

3.3. Glycosidically bound aroma analysis of "Huizao"

The glycosidically bound volatile compounds in jujube were firstly studied. A total of 17 bound volatile compounds were detected in "Huizao", including alcohols (2), aldehydes (1), ketones (1), acids (6), lactones (3), olefins (1), pyrans (1), phenols (2) (**Table 3-4**). With regard to bound volatiles, alcohols were predominant representing 64.90% of the main fraction, followed by acids (20.67%) and phenols (5.37%). This result compared with other fruits, which was close to the 17 bound compounds observed in citrus fruits (Ren et al., 2015b), whereas it was less than baby kiwi (89) (Garcia et al., 2011), strawberry (51) (Ubeda et al., 2012), "Rainer" cherry (54) (Wen et al., 2014b), mandarin fruits peel (56) (Gao et al., 2018), Turkey tomatoes (31) (Özkaya et al., 2018) and tamarillo (49) (Chen et al., 2020).

Table 3-4 Bound volatile compounds identified in'Huizao'jujube.

No	Compounds	LRI[a]	LRI[b]	Identification method	Concentration (mg/kg)	Aroma characteristics[c]
1	Phenylmethanol	1865	1886	MS/RI	1.726±0.271	almond, boiled cherries, floral, moss, roasted bread
2	Butane-2,3-diol	1556	1553	MS/RI/Std	0.051±0.001	fruit
3	Benzaldehyde	1520	1508	MS/RI/Std	0.112±0.018	bitter almond, burnt sugar, cherry, malt
4	3-Hydroxybutan-2-one	1284	1286	MS/RI/Std	0.050±0.013	butter, cream, green pepper, rancid, sweat
5	Acetic acid	1449	1429	MS/RI/Std	0.406±0.020	acid, fruit, pungent, sour, vinegar

6	3-Methyl-butanoic acid	1666	1680	MS/RI/Std	0.007±0.140	cheese, fecal, putrid fruit, rancid, sweat
7	(*E*)-But-2-enoic acid	1745	1750	MS/RI/Std	0.005±0.001	sweet, cream, butter, fat acid, cheese, goat, pungent, rancid
8	Hexanoic acid	1846	1849	MS/RI/Std	0.011±0.000	dust, fat, grass, rancid, sweat
9	*n*-Decanoic acid	2276	2270	MS/RI	0.034±0.159	fat, fruit, metal, wax
10	Dodecanoic acid	2498	2502	MS/RI	0.103±0.033	caramel, cheese, fruit, roasted nut, sweat
11	Oxolan-2-one	1632	1602	MS/RI/Std	0.006±0.013	coconut, coumarin, onion, sweet, warm
12	5-Ethyloxolan-2-one	1694	1736	MS/RI/Std	0.022±0.002	coconut, cream, fruit
13	6-Methyloxan-2-one	1791	1751	MS/RI/Std	0.005±0.010	balsamic, gasoline, plastic, rubber, solvent
14	Styrene	1261	1254	MS/RI/Std	0.047±0.007	caramel, cotton candy, malt, roasted bread, roasted nut
15	3-Hydroxy-2-methylpyran-4-one	1975	1965	MS/RI	0.006±0.017	medicine, phenol, sharp, smoke, spice
16	Phenol	2002	1992	MS/RI	0.012±0.004	-
17	2,4-Di-tert-butylphenol	2381	2321	MS/RI	0.135±0.004	

[a] Linear retention index on DB-Wax columns were determined by *n*-alkanes.
[b] Linear retention index on DB-WAX column from the literature. And "/" indicated the compound lack of reliable linear retention index value in literature.
[c]The aroma characteristics of compounds were obtained from https://www.vcf-online.nl/VcfHome.cfm.

From **Table 3-1 and Table 3-4**, the volatile profiles of free and bound volatiles have some differences: the free volatile compounds are mainly consisted of acids and esters, while the bound volatile showed the highest concentration in alcohols, and most esters existed as free form. In addition, phenylmethanol, 2,4-di-tert-butylphenol, dodecanoic acid, n-decanoic acid, 6-methyloxan-2-one, 3-hydroxy-2-methylpyran-4-one and phenol were only detected in bound volatile compounds, and phenylmethanol had the highest content (1.726 mg/kg) in all bound volatile compounds. Phenylmethanol contributes to the floral aroma, styrene contributes to the balsamic aroma, oxolan-2-one and dodecanoic acid have been described as having fruit odour. *n*-Decanoic acid could contribute to the fat, grass and rancid notes; 3-hydroxy-2-methylpyran-4-one could contribute to the malt, roasted bread and roasted nut notes. As for dodecanoic acid, after hydrolysis, its content (0.103 mg/kg) was higher than its odour threshold (0.004 mg/kg), thus would provide the fruit characteristic to "Huizao". However, the remaining 6 bound compounds' contents were lower than their odour threshold, thus they could hardly contribute to

the overall sensory characteristic of the "Huizao".

Butane-2,3-diol, 3-hydroxy-2-butanone, acetic acid, benzaldehyde, 3-methyl-butanoic acid, 5-ethyloxolan-2-one, (*E*)-but-2-enoic acid, hexanoic acid, oxolan-2-one and styrene were both found in free and bound volatile components, but they contents are different, the butane-2,3-diol was presented in higher amounts in the bound fraction, the content is nearly twice that of free form. Although expect 3-hydroxy-2-butanone, these 9 bound volatile compounds' contents were lower than their odour threshold. It is worth noted that, 3-hydroxy-2-butanone, 5-ethyloxolan-2-one, (*E*)-but-2-enoic acid, hexanoic acid and 3-methyl-butanoic were key aroma-active compounds in "Huizao". The liberation of these five compounds after enzymatic hydrolysis could increase the content of free form and contribute to the sweet, butter, cream, acid and fruit notes. These volatile compounds are expected to make a major contribution to the overall aroma of the "Huizao". The release of these compounds after their hydrolysis could generate, modify or enhance the overall aroma of "Huizao". It is generally accepted that glycosidically bound compounds has a very obvious promotion effect on the aroma of fruits and fruit processed products, which has given us new insight about glycosidic aroma in fruits. In addition, *β*-D-glucose as the most common glycosyl, it would be also released after hydrolyzed and increase the sugar content of the fruit. Furthermore, one or more other sugar groups could be attached to *β*-D-glucose, such as *α*-L-arabinose, *α*-rhamnose, *β*-D-xylose (Sarry & Günata, 2004). These compounds also increase the sugar content after hydrolyzed to change the overall organoleptic perception of the product. In summary, the study on glycosidically bound aroma of jujube is interesting and quite necessary. The flavour quality would be improved of jujube products through adding *β*-glucosidase.

4. Conclusion

Comprehensive investigation on the free and bound volatile compounds of "Huizao" was conducted. Fifteen key aroma-active compounds were identified from 43 free volatile compounds of "Huizao", including 3-hydroxy-2-butanone, butane-2,3-dione, methyl decanoate, ethyl decanoate, methyl dodecanoate, 5-propyloxolan-2-one, 5-butyloxolan-2-one, 2-ethyl-3,5-dimethyl-pyrazine, (*E*)-but-2-enoic acid, hexanoic acid, hexanal, 6-methyl-5-hepten-2-one, 3-oxobutan-2-yl acetate, 5-ethyloxolan-2-one, and 3-methyl-butanoic acid. In addition, 17 bound volatile

compounds were identified in which phenylmethanol had the highest content. Moreover, as the key aroma-active compounds in “Huizao”, 3-hydroxy-2-butanone, 5-ethyloxolan-2-one, (*E*)-but-2-enoic acid, hexanoic acid and 3-methyl-butanoic were also existed in bound fraction. They would enhance the aroma and contribute to the sweet, butter, cream, acid and fruit notes after enzymatic hydrolysis. The research on the above volatile compounds newly identified as key aroma-active compounds and bound volatile compounds in “Huizao”, would help to comprehensively understand the aroma characteristic of “Huizao”.

4

Chapter IV. Key aroma-active compounds identification of *Ziziphus jujuba* cv. Huizao: Effect of pilot scale freeze-drying

Ziziphus jujuba cv. Huizao is one of the most popular cultivars, which is mainly used for drying. With the development of freeze drying, freeze-dried red jujube become popular. However, aroma has been reported to be lost after freeze drying at laboratory scale. In actual industry production, multi-stage and variable-temperature procedure was used with a higher temperature. The aim of this chapter was to investigate the effect of pilot scale freeze drying on the aroma profile of red jujube, and identify key aroma-active compounds of freeze-dried red jujube.

Gou, M., Chen, Q., Qiao, Y., Jin, X., Zhang, J., Yang, H., Fauconnier, M. L., & Bi, J. (2023). Key aroma-active compounds identification of *Ziziphus jujuba* cv. Huizao: Effect of pilot scale freeze-drying. Journal of Food Composition and Analysis, 116(November 2022), 105072. https://doi.org/10.1016/j.jfca.2022.105072

Abstract: Aroma profile and key aroma-active of red jujube subjected to pilot scale freeze drying (FD) were investigated based on molecular sensory science. After FD, 41 aroma compounds were identified through gas chromatography-olfactometry-mass spectrometry (GC-O-MS) and quantified through calibration curves. The total aroma compounds content was decreased 26.71% compared with raw red jujube, of which ketones and acids contents were decreased 63.33% and 62.88%, while, the esters, lactones and alcohols contents were increased 34.10%, 8.52% and 480.17%, respectively. Through the GC-O-MS, odour active values combined with recombination and omission tests, 14 key aroma-active compounds were identification in freeze-dried red jujube. In which, 2-ethyl-3,5-dimethyl-pyrazine had the highest OAV (2,687.00) and dominated the roast note of aroma profile. In addition, ethyl heptanoate, hexyl acetate, 2,3-butanediol, ethyl dodecanoate and 5-heptyloxolan-2-one were newly identified as the key aroma-active compounds in freeze-dried red jujube.

Keywords: red jujube, GC-O-MS, omission tests, odour activity values, detection frequency

1. Introduction

Jujube (*Zizyphus jujuba* Mill.) is a plant of the family Rhamnaceae and originated in China with a long history of more than 4000 years (Qinqin Chen et al., 2018). As a characteristic resource of homology of medicine and food in China, it has high biological activities, such as sedation, tranquilization, blood enrichment, brain strengthening, and anti-cancer (Choi et al., 2012). In addition, it has a pleasant aroma, which is one of the important factors to attract consumers and directly affects its commodity value. *Ziziphus jujuba* cv. Huizao is one of the most popular cultivars, which is mainly used for drying. Freeze drying (FD) is a drying method with direct dehydration of frozen materials under reduced pressure. This process could better retain the color, shape, texture and nutritional of raw materials, and it is more and more widely applied in the food production, such as, fruit and vegetable crisps, coffee powder and yogurt cubs (An et al., 2016; W. Xu et al., 2021).

Aroma is an important characteristic of dried fruit products, however, most freeze-dried products showed different degrees of aroma loss. The changes of aroma compounds in freeze-dried process of different materials have attracted the attention of many researchers. To date, Song et al. (2020) studied the volatile compounds in red jujube subjected to different drying processes, the aldehydes compounds in red jujube were no longer detected after FD. Chin et al. (2008) studied the changes in aroma of durian after FD for 12 h, the amount of major aroma decreased dramatically, ranging from 71% to 97%, and most of them were esters. Rajkumar et al. (2017) compared the volatile components between fresh cabbage and the freeze-dried one, found aldehydes, esters, alcohols, and ketones had a certain degrees of loss. Similar aroma loss were also observed in freeze-dried tomato (Jeyaprakash et al., 2020), garlic (Feng et al., 2021), golden pompano (Zhang et al., 2019), banana (Mui et al., 2002) and bread crumbs (Dimelow et al., 2005), the average loss ratio of volatile compounds of freeze-dried banana slices and carvone in bread crumbs was 37.5% and 55%, respectively. The causes of aroma loss are complex in freeze-dried process, especially in the last stage of FD, the temperature of the material would be close to the heating plate, and the volatile compounds might be transformed or migrated. Else, some volatile compounds, which have the low vapor pressure, would be combined with the sublimated water vapor in the cold trap of the freeze dryer. Some volatile compounds condensed and discharged by the vacuum pump, so the change of the volatile compounds of the freeze-dried products is a very complicated

physical and chemical process, which is closely related to the drying process condition (Q. Yan et al., 2019).

However, lower constant freeze-drying temperature (less than 30 °C) and experimental FD machine were usually used in the published literature, which showed significant difference with the one used in the industry. To improve productivity and reduce energy consumption, multi-stage and variable-temperature procedure was used in industry with a higher temperature of heating plate (85~65 °C) to provide higher latent heat for the sublimation of water in the material, and accelerate the FD rate and shorten the FD time. In this process, the temperature of the material will gradually increase from the freezing temperature (-40 °C) to the heating plate temperature (65 °C). In the later desorption drying stage, chemical reactions will occur to enhance the aroma of products, which might offset the aroma loss in experimental FD. Fortunately, drying conditions used in the industry FD could be well simulated by pilot scale FD, which could be used for better understanding aroma change of jujube during multi-stage and variable-temperature procedure.

To understand how the jujube aroma was modified after FD, the effect of pilot scale FD on the aroma profile of red jujube was first analyzed, and key aroma-active compounds were further identified based on molecular sensory science and technology. It could provide theoretical basis for aroma quality improvement during FD process.

2. Materials and methods

2.1. Materials and chemicals

Red jujubes (*Zizyphus jujuba* cv. Huizao) were harvested from a local farm orchard in Akesu, Xinjiang, China, in November 2020. The red jujubes were selected and transported to Beijing within 2 days. The red jujubes without any damage and incubated at 4 ^{o}C controlled atmosphere storage room until used. The water content of “Huizao” was 25.57%, the pH was 5.5, and the solid soluble content was 69.0%.

Oct-1-en-3-ol , 2,3-butanediol, hexanal, (*E*)-2-hexenal, nonanal, furfural, 2-octenal, decanal, benzaldehyde, butane-2,3-dione, 3-octanone, 3-hydroxybutan-2-one, oct-1-en-3-one, 6-methyl-5-hepten-2-one, 3-oxobutan-2-yl acetate, undecan-2-one, acetic

acid, 3-methyl-butanoic acid, pentanoic acid, (*E*)-but-2-enoic acid, hexanoic acid, heptanoic acid, (*E*)-2-hexenoic acid, octanoic acid, methyl hexanoate, hexyl acetate, ethyl heptanoate, methyl decanoate, ethyl decanoate, methyl benzoate, methyl dodecanoate, ethyl dodecanoate, hexamethyl decanoate, oxolan-2-one, 5-ethyloxolan-2-one, 5-propyloxolan-2-one, 5-butyloxolan-2-one, 5-heptyloxolan-2-one, limonene, γ-terpinene, styrene, α-farnesene, 2-pentyl-furan, p-cymene, naphthalene, 2-ethyl-3,5-dimethyl-pyrazine, 2,6-dimethyl-pyrazine, tetramethyl-pyrazine and 2-ethyl-6-methyl-pyrazine were purchased from Yuanye Bio-Technology (Shanghai Yuanye Bio-Technology Co., Ltd) or Macklin (Shanghai Macklin Biochemical Co., Ltd). All of the chemical standards used above with purity ≥ 99%.

2.2 Freeze drying (FD) treatment

Briefly, the kernel of jujubes was removed and the remaining part was cut into 5 mm slices; then 500 g jujube slices soaked in 80 ℃ water for 1 min to keep a relatively flat surface before freeze drying. Jujube slices were drained in a colander and put into -40 °C refrigerator for 48 h. A pilot scale freeze dryer (Advantech Co., Ltd. China) was used with drying conditions was set as follows: The cold trap temperature and vacuum pressure were -30 °C and 60 Pa, respectively; the drying temperature of the heating plate was from room temperature (25 °C) to 85 °C within 45 min and kept for 3 h, then decreased to 70 °C within 30 min and maintained for 5 h, and finally decrease to 65 °C within 30 min and kept for 1 h. The sample tray was in the middle of two heating plates and not directly connected, the diagram was shown in **Figure 4-1**. At the same time, the temperature of jujube slices was monitored online through the temperature probe which equipped in FD machine (**Figure 4-2**).

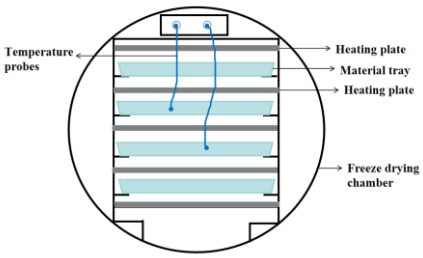


Figure 4-1 The diagram of drying chamber in pilot scale freeze dryer.

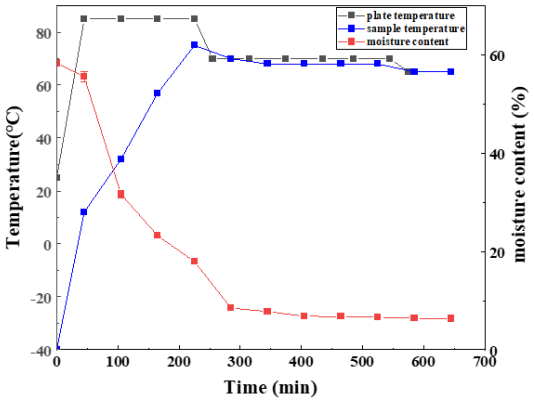


Figure 4-2 The changes of plate temperature, sample temperature and moisture content of red jujube during freeze drying processing.

2.3 Extraction volatile compounds from jujube by headspace solid-phase microextraction (HS-SPME)

HS-SPME was carried out according to Gou et al., (2022) and Qiao et al., (2021). Briefly, the kernel of jujubes was removed and the remaining part was cut into 5 mm slices; then crushed using a Joyoung pulverizer (JYL-CO20, Joyoung Co., Ltd., Shandong, China). The 2 g of the sample were placed in 20-mL headspace vials, and 2 μL of 2-cyclohexen-1-one (1 mg/mL) and 0.1 g NaCl in 0.5 mL of distilled water was added, followed by incubation at 50 °C for 40 min to reach equilibrium. A resolved SPME fiber (polydimethylsiloxane-divinylbenzene (PDMS/DVB), 2 cm, 65 μm; Supelco, Inc., Bellefonte, PA, USA) was placed in the sample headspace, and adsorption was carried out for 30 min at 50 °C. After extraction, the SPME fiber was withdrawn and directly insert into the GC injector for desorbing at 250 °C for 3 min. The experiment was repeated at least three times independently for each sample.

2.4 Gas chromatography-olfactometry-mass spectrometry (GC-O-MS) analysis

2.4.1. Detection of volatile compounds

The volatile compounds of the sample were identified by GC-MS (7890B GC System, 5977A MSD) equipped with sniffing port (Sniffer 9000, Brechbühler, Schlieren, Switzerland) and a DB-Wax column (60 m×0.25 mm, 0.25 μm). The temperature programs were designed as follows: the column temperature was held

40 °C for 3 min, heated to 90 °C at 7 °C /min, increased to 120 °C at 4 °C /min, then rose to 170 °C at 5 °C/min, thereafter, ramped to 200 °C at 4 °C/min, and held for 8 min. The helium carrier gas (purity = 99.99%) was input at a constant flow rate of 1.0 mL/min. The ionization method was Electron-impact (EI), and the fragments created by EI were scanned from 35 to 550 m/z.

A sniffing port (Sniffer 9000) coupled to the GC-MS instrument was used to discriminate the aroma-active compounds in the HS-SPME isolates. At the end of the capillary column, the effluent between the olfactometers (Sniffer 9000, Brechbühler, Schlieren, Switzerland) and the MS detector was split at ratios of 1:1, of which one was delivered to the sniffing port (280 °C), and the other was delivered to the MS.

2.4.2 Qualification of volatile compounds

The aroma compounds were identified by comparing the NIST17 library of the MS and were confirmed by the linear retention indices (LRI), odour qualities and authentic standards. A total of 3 trained sensory evaluators were selected to smell the aroma compounds in the samples by olfactometry. These professionals had outstanding sensory organs, no bad habits, and no allergies. The evaluators recorded the odour characteristics and detection frequency (DF) of the aroma compounds in the samples. DF analysis was repeated twice for every panelist. Odourant with DF $\geq$ 2 (reported by at least two assessors) could be considered as having aroma potential activity. Authentic flavour standards were also used to confirm the volatile compounds as external references under same GC-O-MS conditions.

2.4.3 Quantification of volatile compounds

All aroma compounds were quantified by calibration curves constructed with authentic flavour standards in the odourless matrix. To obtain an odourless matrix, the aroma compounds of samples were removed by a mixture of ether and pentane (v:v = 1:1). Subsequently, the samples were dried at 20 °C for 48 h using a freeze dryer (Alpha 1-4 LD plus, Marin Christ, Osterode, Germany) until nothing was detected by GC-O-MS (Liu et al., 2020).

The extraction method and GC-MS condition of standard volatiles for preparation of standard curves as same as described for the analysis above mentioned. The standard curves for different compounds (R^2 > 0.99) were established. The calculation of each identified compound was according to the standard curve and results were shown in **Table 4-1**.

Table 4-1 Calibration equations and coefficients of determination (R^2) of volatile compounds in raw and vacuum freeze-dried red jujube.

No.[a]	Compounds	Calibration equations[b]	R^2
A1	Oct-1-en-3-ol	y=9.708x+0.152	0.999
A2	2,3-Butanediol*	y= 107421.9x-343625.6	0.963
B1	Hexanal	y=1.065x+0.031	0.988
B2	(*E*)-2-Hexenal	y = 0.822x -0.017	0.989
B3	Nonanal	y=0.856x+0.033	0.998
B4	2-Octenal	y=2.833x+0.044	0.998
B5	Furfural	y=0.331x+0.002	0.999
B6	Decanal	y=1.704x+0.016	0.999
B7	Benzaldehyde	y=2.237x+0.064	0.999
C1	Butane-2,3-dione	y = 0.133x-0.242	0.979
C2	3-Octanone	y=12.393x+0.010	0.999
C3	3-Hydroxybutan-2-one	y = 0.060x +0.142	0.980
C4	Oct-1-en-3-one	y=2.221x+0.012	0.989
C5	6-Methyl-5-hepten-2-one	y=6.005x+0.041	0.999
C6	3-Oxobutan-2-yl acetate	y=0.002x+0.010	0.966
C7	Undecan-2-one	y=1.914x+0.030	0.995
D1	Acetic acid*	y= 813986x-6E+06	0.995
D2	3-Methyl-butanoic acid	y = 0.034x+0.014	0.955
D3	Pentanoic acid	y = 0.067x+0.007	0.980
D4	(*E*)-But-2-enoic acid	y=0.067x+0.007	0.980
D5	Hexanoic acid	y = 0.746x-0.044	0.996
D6	Heptanoic acid	y=1.202x-0.036	0.998
D7	(*E*)-2-Hexenoic acid	y=0.007x-0.005	0.968
D8	Octanoic acid	y=0.207x+0.063	0.997
E1	Methyl hexanoate	y=1.037x+0.035	0.967
E2	Hexyl acetate	y=0.460x-1.778	0.993
E3	Ethyl heptanoate	y=0.059x-0.009	0.910
E4	Methyl decanoate	y=84.091x+0.845	0.999
E5	Ethyl decanoate	y=6.976x-0.220	0.996
E6	Methyl benzoate	y=1.633x+0.144	0.993
E7	Methyl dodecanoate	y=102.655x+0.652	0.998
E8	Ethyl dodecanoate	y=8.081x-0.317	0.993
F1	Oxolan-2-one	y=0.029x+0.003	0.999
F2	5-Ethyloxolan-2-one	y=0.348x+0.002	0.999
F3	5-Propyloxolan-2-one	y=0.143x+0.018	0.999
F4	5-Butyloxolan-2-one	y=1.084x+0.001	0.999
F5	5-Heptyloxolan-2-one	y=9.150x-0.435	0.999
G1	2,6-Dimethyl-pyrazine	y=0.458x+0.005	0.999
G2	2-Ethyl-6-methyl-pyrazine	y=2.331x+0.015	0.999
G3	2-Ethyl-3,5-dimethyl-pyrazine	y=1.467x-0.014	0.999
G4	Tetramethyl-pyrazine	y= 0.786x+0.044	0.999
H1	Limonene	y=9.267x+0.165	0.995
H2	γ-Terpinene	y= 0.010x-0.003	0.992
H3	Styrene	y=9.731x+0.078	0.996

H4	α-Farnesene	y= 0.007x-0.005	0.990
I1	2-Pentyl-furan	y= 14.683x-0.245	0.999
I1	*p*-Cymene	y=0.025x+0.001	0.993
I2	Naphthalene	y=29.367x+0.084	0.998

[a]The numbers assigned to the compounds are consistent with those in **Table 4-2**. [b]Variables: y is the peak area relative to that of the internal standard, 2-cyclohexen-1-one, and x is the concentration ($\mu g kg^{-1}$) in the jujube sample relative to that of the internal standard, 2-cyclohexen-1-one. *: y is the peak area of compounds, and x is the concentration ($\mu g kg^{-1}$) of the jujube sample in 2,3-butanediol and acetic acid.

2.5. Odour Activity Value (OAV)

To obtain information of the importance of a single compound in the aroma profile of red jujube, the OAV was calculated, which is the ratio of the concentration of each compound to its orthonasal detection odour threshold. The threshold values of volatile compounds in water referred to the literature (Gemert, 2011).

2.6. Aroma recombination and omission experiments

To identify the key aroma-active compounds based on GC-O-MS and OAV results, recombination and omission were carried out. Each omission sample was evaluated against complete recombination model prepared by mixing the standard aroma compounds at the concentrations in freeze-dried jujube (recombination model 1). If there is a significant difference, it means that the missing aroma component is the key aroma-active compound of sample. On this basis, the key aroma-active compounds were selected for the recombination test (recombination model 2). Sensory evaluation analyzes the similarity of the odour between the recombinant model 2 and freeze-dried jujube, and determines the aroma characteristics of freeze-dried jujube. In the end, this study selected 10 sensory evaluators (23-27 years old, 4 males and 6 females) who were experienced and engaged in food flavour research for sensory evaluation. The analysis was according to Gou et al., (2022) .

2.7. Quantitative descriptive analysis

The quantitative descriptive analysis (QDA) was often applied to describe the aroma profiles of samples. In this study, 3-hydroxybutan-2-one for “cream” note, 5-butyloxolan-2-one for “floral” note, hexanal for “green” note, methyl dodecanoate for “fruity” note, 2-ethyl-3,5-dimethyl-pyrazine for “roast” note, and (*E*)-but-2-enoic acid for “sweet” note, acetic acid for “sour” note, 3-methyl-butanoic acid for “rancid” note and 2-ethyl-6-methyl-pyrazine for “nut” note were the reference compounds of aroma descriptors which were dissolved in water at a concentration of

100 times of their respective odour threshold (Zhang et al., 2021). QDA was conducted in triplicate by same panelists above mentioned. Scores for each sample in 0.5 increments, from 0.0 to 3.0 on the basis of 7-point scales (0, none; 1.5, moderate; and 3, very strong) (Gou et al., 2022).

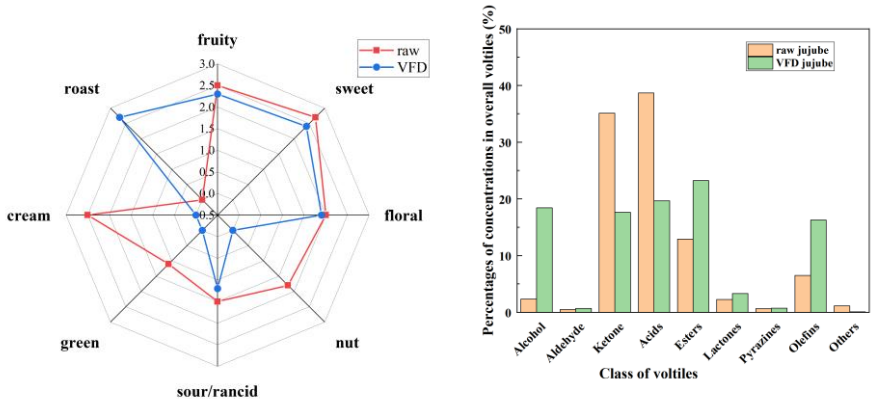
2.8. Statistical Analysis

All statistical analyses were performed using SPSS version 20.0 software (SPSS Inc., Chicago, IL). Contents of different components were presented as the mean ± SD (standard deviation). The data were illustrated using Origin 2018 (OriginLab Corporation, Northampton, MA). The venn graph was performed by http://jvenn.toulouse.inra.fr/app/example.html.

3. Results and discussion

3.1 Effect of pilot scale FD treatment on aroma properties of red jujube

QDA was performed to evaluate the aroma profile of freeze-dried sample. As shown in **Figure 4-3(a),** there were only five major aroma attributes of freeze-dried red jujube, namely roast, sour/rancid, sweet, fruity and floral. Compared with the raw red jujube, significant change of the aroma characteristics was observed after FD. As for the overall aroma profile, the score of sweet (2.7), fruity (2.5) and cream (2.5) notes were higher than others, dominated aroma notes in raw red jujube. However, the roast note (2.7) exhibited the highest score compared with other aroma attributes in freeze-dried samples. That may be due to the accumulation of pyrazines in FD processing which present the roast and bake notes. Green and cream notes were not observed in the freeze-dried red jujube, which could be mainly interpreted by the loss of C6 aldehydes and ketones during FD processing (**Figure 4-3(b)**). The sour/rancid note exhibited the weak intensity (1.2) in the freeze-dried sample because of the high loss ratio of acids (62.88%). Besides, the score of sweet, fruity and floral had less change after FD (**Figure 4-3(b)**).



(a) (b)

Figure 4-3 Volatile profiles (a), content percentages of the major odour classes (b) in raw and freeze-dried jujube.

3.2 Effect of pilot scale FD treatment on volatile compounds of red jujube

A total of 41 volatile compounds were identified in pilot scale FD red jujube, including aldehydes (4), alcohols (2), acids (7), esters (9), ketones (3), lactones (5), pyrazines (4), olefins (4), *p*-cymene, naphthalene and 2-pentyl-furan (**Table 4-2**). The amount of volatile compounds after FD (41) was not changed obviously compared with raw jujube (42), and the content was only reduced by 26.71%, which represent a higher volatile compounds retention than previously reported literature (An et al., 2016; Mui et al., 2002; Rajkumar et al., 2017). That might be due to the higher temperature of heating plate in this study. Some volatile can be produced by chemical reaction, which can offset the aroma loss during freeze drying. In previous study, the condition of freeze drying had a lower temperature of plate (20 ℃ for banana chips, 25 ℃ for ginger slices and 45 ℃ for cabbage leaves, until they were dried) (An et al., 2016; Mui et al., 2002; Rajkumar et al., 2017).

However, the volatile compounds composition changed significantly after FD. The distribution of these volatile compounds was presented in the **Figure 4-3(b)**, the proportions of acids and ketones of freeze-dried red jujube decreased (19.61% and 17.60% of the total volatiles) in comparison with those in the raw red jujube. Moreover, the proportions of esters (23.16%), alcohols (18.37%) and pyrazines

(0.94%) increased.

Table 4-2 Contents of volatile compounds in raw and freeze-dried jujube.

No	Compounds	Contents(μg/kg)	
		Raw jujube	VFD-jujube
Alcohol			
A1	Oct-1-en-3-ol	0.63±0.05	20.45±0.17
A2	2,3-Butanediol	799.97±18.44	4624.43±139.12
total		800.60	4644.88
Aldehyde			
B1	Hexanal	17.07±0.00	24.02±0.11
B2	(*E*)-2-Hexenal	32.12±1.26	nd
B3	Nonanal	nd	10.88±1.07
B4	2-Octenal	0.34±0.03	nd
B5	Furfural	37.23±2.05	135.93±2.17
B6	Decanal	0.47±0.02	nd
B7	Benzaldehyde	80.05±5.10	1.86±0.05
total		167.29	172.68
Ketone			
C1	Butane-2,3-dione	2388.62±80.32	nd
C2	3-Octanone	1.07±0.04	1.41±0.04
C3	3-Hydroxybutan-2-one	9242.39±205.87	3266.01±94.89
C4	Oct-1-en-3-one	0.12±0.01	nd
C5	6-Methyl-5-hepten-2-one	0.88±0.02	nd
C6	3-Oxobutan-2-yl acetate	497.90±18.93	1178.03±87.84
C7	Undecan-2-one	nd	2.66±0.07
total		12130.99	4448.10
Acids			
D1	Acetic acid	3549.99±93.54	1920.65±84.26
D2	3-Methyl-butanoic acid	1952.98±79.19	1792.30±101.29
D3	Pentanoic acid	nd	505.98±4.72
D4	(*E*)-But-2-enoic acid	1204.87±35.81	505.52±4.72
D5	Hexanoic acid	2180.26±66.80	92.71±3.72
D6	Heptanoic acid	158.67±11.02	93.36±9.03
D7	(*E*)-2-Hexenoic acid	4040.63±36.73	nd
D8	Octanoic acid	271.49±27.02	47.88±1.31
total		13358.89	4958.40
Esters			
E1	Methyl hexanoate	135.43±10.94	203.61±1.11
E2	Hexyl acetate	3900.47±5.67	3875.27±2.55
E3	Ethyl heptanoate	258.29±18.64	911.18±3.76
E4	Methyl decanoate	3.79±0.33	27.48±0.29
E5	Ethyl decanoate	31.61±0.02	350.95±0.06
E6	Methyl benzoate	79.12±7.12	93.09±0.81
E7	Methyl dodecanoate	5.38±0.26	20.25±0.64
E8	Ethyl dodecanoate	nd	349.02±0.01
E9	Methyl hexadecanoate	41.05±2.68	23.39±2.06

total		4455.14	5854.24
Lactones			
F1	Oxolan-2-one	456.31±17.30	519.52±27.02
F2	5-Ethyloxolan-2-one	202.04±0.99	91.72±9.08
F3	5-Propyloxolan-2-one	86.21±1.46	170.38±2.79
F4	5-Butyloxolan-2-one	28.29±0.31	9.19±0.08
F5	5-Heptyloxolan-2-one	nd	47.85±0.26
total		772.85	838.66
Pyrazines			
G1	2,6-Dimethyl-pyrazine	0.22±0.00	1.33±0.02
G2	2-Ethyl-6-methyl-pyrazine	16.80±1.37	13.64±0.99
G3	2-Ethyl-3,5-dimethyl-pyrazine	88.00±7.84	107.48±0.20
G4	Tetramethyl-pyrazine	116.01±0.34	63.13±0.95
total		168.15	238.45
Olefins			
H1	Limonene	1.07±0.07	0.34±0.04
H2	*γ*-Terpinene	nd	1519.69±66.31
H3	Styrene	0.89±0.03	9.67±0.08
H4	*α*-Farnesene	2245.23±91.23	2572.99±166.27
total		2247.19	4102.69
Others			
I1	2-Pentyl-furan	nd	18.07±0.02
I2	*p*-Cymene	389.50±22.39	nd
I3	Naphthalene	0.11±0.01	3.05±0.02
total		389.62	21.12
Total		**34490.71**	**25279.23**

nd: not detected.

Esters, related to fruity characteristics (Gou et al., 2021), were found to increase after FD. As shown in **Table 4-2,** methyl hexanoate and ethyl heptanoate, featured fruity odourant, ranged widely from 135 μg/kg (raw) to 203 μg/kg (FD), and 258 μg/kg (raw) to 911 μg/kg (FD), respectively. And ethyl dodecanoate was newly detected after FD. Besides, methyl decanoate, ethyl decanoate and methyl dodecanoate were increased obviously. These compounds have been reported to be important contributor to the aroma of "Huizao" jujube (Gou et al., 2022). However, no significant differences were found between raw and freeze-dried samples for the concentrations of hexyl acetate and methyl benzoate. The increased content of ester compounds are generally formed through non-enzymatic esterification of alcohols and organic acids, contributed the fruity note (Y. Wang et al., 2020).

Alcohols were increased notably after FD (from 800 μg/kg to 4644 μg/kg), which might be caused by the glucose metabolism, amino acid decarboxylation and dehydrogenation with prolonged FD time or oxidation and degradation of

polyunsaturated fatty acids (Ye et al., 2022). Among them, oct-1-en-3-ol with typical mushroom note, which might be generated from oxidation of fatty acids (W. Zhu et al., 2019) and 2,3-butanediol, related with creamy, floral and fruity notes, which might be originated from metabolism of pyruvate (Zhang et al., 2020).

Acids content showed a decreasing tendency after FD treatments, which was decreased 62.88% compared with raw jujube. That might be due to the occurrence of chemical reaction, leading the acids transformed to esters through the multi-stage and higher temperature applied in this study. (*E*)-2-Hexenoic acid is the most abundant volatile acid, while, it was not detected in freeze-dried red jujube. That might be discharged by vacuum pump, due to its volatility. Acetic acid also had an obvious loss rate (45.90%), which might be carried away by the water vapor because of its polarity, or involved in the Maillard reaction. Moreover, acids compounds having usually high thresholds, will contribute slightly to the overall aroma of freeze-dried red jujube.

The content of ketone compounds also obviously decreased after FD, with the loss ratio of 63.33%. The content of 3-hydroxybutan-2-one with cream note decreased 64.66%. Especially, butane-2,3-dione with cream note and 6-methyl-5-hepten-2-one with sweet note were key aroma-active compounds in raw red jujube (Gou et al., 2022), but they did not detected after FD, which caused the loss of "cream" and "sweet" aroma properties of freeze dried red jujube. Generally, most of ketones produced by reducing sugars and amino acid degradation or unsaturated fatty acid oxidation (Zhang et al., 2019). These ketones as α-dicarbonyl compounds could participate in the Maillard reaction and form pyrazines, such as butane-2,3-dione and 3-hydroxybutan-2-one (Mei et al., 2007; Xiao et al., 2018).

Pyrazines are generally results from the Maillard reaction, which are more favorable at high temperature. The percentage of pyrazines content (0.94%) in FD was almost two times higher than in the raw jujube (0.49%). Pyrazines contributed to important aroma characteristics due to their low thresholds, especially 2-ethyl-3,5-dimethyl-pyrazine, which was the major contributor of nut notes in red jujube (Gou et al., 2022). Meanwhile, the increase of pyrazine content may also lead to the enhancement of roast note of freeze-dried red jujube. The alkylpyrazine was produced by condensation of carbonyl compounds, which are degradation products from reducing sugars. The α-carbonyl compounds could react with amino acids to form α-aminocarbonyl compounds and Strecker aldehydes, furthermore, converted

to corresponding alkylpyrazine (Deng et al., 2022; Guerra & Yaylayan, 2012; Mei et al., 2007; Scalone et al., 2015). Amino acids and reducing sugars were existed in red jujubes provided good resource for the formation of pyrazine compounds (J. Song et al., 2019).

Six and four aldehydes were quantified in raw and freeze-dried samples, respectively. Though the number of aldehyde compounds was decreased, the content of aldehyde was slightly increased in freeze-dried jujube. That might be due to oxidative degradation of aroma precursors, such as fatty acids (Yu Zhao et al., 2021). The furfural was increased remarkably, because in the later stage of FD, the red jujube temperature gradually close to the heating plate temperature (65 ℃), resulted the degradation of carbohydrates or Maillard reaction (H. Y. Yu et al., 2020). Five lactones compounds were detected in the freeze-dried samples which could be generated as a result of hydroxyl acid intramolecular esterification (Al-Dalali et al., 2020) or formed from the reaction between glycine and D-glucose in slightly acidic (Keyhani & Yaylayan, 1996). All of the detected lactone compounds were found in both raw and freeze-dried samples, except for 5-heptyloxolan-2-one, which was only detected in freeze-dried samples. Olefins contents were increased sharply in freeze-dried red jujube, and γ-terpinene was newly detected. In addition, p-cymene and naphthalene were quantified in raw samples, and 2-pentyl-furan and naphthalene were quantified in freeze-dried samples. However, the concentrations of these compounds were low in both samples.

3.3 Identification of aroma-active compounds in pilot scale FD red jujube by GC-O-MS analysis

DF was widely used as the recognition for aroma intensity of the aroma compound. The compound perceived more times by the evaluators, it could be considered to have a larger importance (Gou et al., 2021). There were alcohol (1), aldehydes (2), ketones (5), acids (5), esters (2), lactones (3), pyrazines (2) and olefin detected by DFA in raw red jujube. While, there were alcohols (2), aldehydes (1), ketones (2), acids (5), esters (4), lactones (4) and pyrazines (3) in FD red jujube (**Table 4-2**). Although the amount aroma-active compounds was 21 in both raw and freeze-dried samples (**Table 4-3 and Figure 4-4**), the compositions of aroma-active compounds were quite different. In freeze-dried sample, 14 aroma-active compounds also recognized in raw jujube, and 7 compounds were newly detected as aroma-active compounds, such as 2,3-butanediol, octanoic acid, ethyl dodecanoate, 5-

heptyloxolan-2-one, 2,6-dimethyl-pyrazine and tetramethyl-pyrazine. Furthermore, roast-like 2-ethyl-3,5-dimethyl-pyrazine and fruit-like ethyl heptanoate were perceived by all assessors (DF=6), as well as oct-1-en-3-ol with mushroom and floral notes, 3-hydroxybutan-2-one with sweet and fat notes, 3-oxobutan-2-yl acetate with sweet, fat notes, methyl dodecanoate with coconut, fruit and sweet notes, and fruity-like ethyl decanoate, revealing that they had major contributions to the aroma profile of freeze-dried red jujube. In addition, 2,3-butanediol (fruity), 5-butyloxolan-2-one (fruity) and 5-heptyloxolan-2-one (sweet, coconut) were also recognized their moderate contributions to the aroma of freeze-dried red jujube, because of their relatively higher DF ($6 > DF \geq 4$). The other 9 compounds in freeze-dried red jujube, benzaldehyde (bitter almond), hexanoic acid (acid), 3-methyl-butanoic acid (rancid), (*E*)-but-2-enoic acid (sweet), ethyl dodecanoate (floral, fruit), 5-ethyloxolan-2-one (sweet), 5-propyloxolan-2-one (sweet), 2,6-dimethyl-pyrazine (baked), tetramethyl-pyrazine (roast)) were considered as the potent contributors in the aroma profile of FD red jujube.

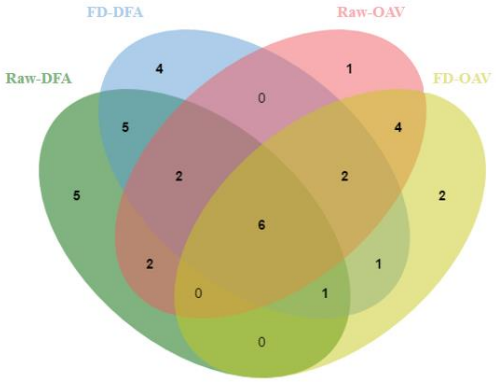


Figure 4-4 The venn diagram of aroma-active compounds identified in raw and freeze-dried jujube by OAV and DF.

In general, alcohols and aldehydes were perceived as floral and green odours, esters contributed fruity odour, acids mainly related to sweat and sour notes, ketones offered cream and sweet aroma, lactones described as sweet and fruity notes, while pyrazines contributed a nut or roast note. In raw red jujube, ketones identified as the dominant aroma-active compounds by DF, thus the cream note is the one of the characteristic aroma in raw jujube. While, roast and more intense fruity aroma

characteristics performed in freeze-dried jujube, owing to the main aroma-active compounds were changed into esters and pyrazines after FD. These results are consistent with above mentioned sensory evaluation results.

Table 4-3 Aroma-active compounds in raw and freeze-dried jujube obtained from OAV and DFA

No	Compounds	LRI[a]	LRI[b]	Identification method[c]	DF[d]		OAV[e]		Odor description[f]
					Raw jujube	FD-jujube	Raw jujube	FD-jujube	
Alcohol									
A1	Oct-1-en-3-ol	1450	1448	MS/O/RI/Std	6	6	0.42±0.03	13.63±0.11	earth, fat, floral, mold, mushroom
A2	2,3-Butanediol	1556	1553	MS/O/RI/Std	<2	4	8.41±0.19	48.63±1.46	fruit
Aldehyde									
B1	Hexanal	1083	1078	MS/RI/Std	<2	<2	3.41±0.00	4.80±0.02	
B2	(*E*)-2-Hexenal	1216	1228	MS/RI/Std	<2	nd	0.29±0.01	nd	
B3	Nonanal	1391	1409	MS/RI/Std	nd	<2	nd	9.89±0.97	
B4	2-Octenal	1429	1410	MS/O/RI/Std	2	nd	0.11±0.01	nd	green, nut, fat
B5	Furfural	1460	1479	MS/RI/Std	<2	<2	0.04±0.00	0.01±0.00	
B6	Decanal	1480	1498	MS/RI/Std	<2	nd	0.16±0.01	nd	
B7	Benzaldehyde	1520	1508	MS/O/RI/Std	4	2	0.11±0.01	0.00±0.00	bitter almond, burnt sugar, cherry, malt
Ketone									
C1	Butane-2,3-dione	997	979	MS/O/RI/Std	6	nd	2388.62±80.32	nd	butter, caramel, cheese, cream, fruit
C2	3-Octanone	1253	1240	MS/RI/Std	<2	nd	0.05±0.00	0.07±0.00	
C3	3-Hydroxybutan-2-one	1284	1286	MS/O/RI/Std	4	6	660.17±14.71	233.29±6.78	butter, cream, green pepper, sweat
C4	Oct-1-en-3-one	1290	1296	MS/RI/O/Std	4	nd	40.00±3.33	nd	earth, green, metal, mushroom
C5	6-Methyl-5-hepten-2-one	1338	1342	MS/O/RI/Std	4	nd	0.01±0.00	nd	citrus, mushroom, rubber, strawberry
C6	3-oxobutan-2-yl acetate	1378	/	MS/O/RI/Std	6	6	-	-	cream, sweet, fat
C7	Undecan-2-one	1598	1615	MS/RI/Std	nd	<2	nd	0.48±0.01	
Acids									

D1	Acetic acid	1449	1429	MS/O/RI/Std	<2	<2	0.04±0.00	0.02±0.00	
D2	3-Methyl-butanoic acid	1666	1680	MS/O/RI/Std	2	2	3.99±0.16	3.66±0.21	cheese, fecal, putrid fruit, rancid, sweat
D3	Pentanoic acid	1733	1762	MS/RI	nd	<2	nd	0.05±0.00	
D4	(*E*)-But-2-enoic acid	1745	1750	MS/O/RI/Std	2	4	-	-	sweet
D5	Hexanoic acid	1846	1849	MS/O/RI/Std	3	3	2.45±0.08	0.10±0.00	acid, cheese, goat, pungent, rancid
D6	Heptanoic acid	1950	1943	MS/O/RI/Std	2	5	0.25±0.02	0.15±0.01	apricot, floral, rancid, sour, sweat
D7	(*E*)-2-Hexenoic acid	1967	1994	MS/O/RI/Std	2	nd	-	nd	fat, must
D8	Octanoic acid	2060	2086	MS/O/RI/Std	<2	4	0.09±0.01	0.16±0.00	acid, cheese, fat, rancid, sweat
Esters									
E1	Methyl hexanoate	1184	1177	MS/RI	<2	<2	1.93±0.16	2.91±0.02	
E2	Hexyl acetate	1272	1265	MS/RI	<2	<2	34.21±0.05	33.99±0.02	
E3	Ethyl heptanoate	1326	1342	MS/O/RI/Std	<2	6	135.94±9.81	479.57±1.98	brandy, fruit, wine
E4	Methyl decanoate	1593	1636	MS/RI/Std	<2	<2	1.08±0.08	6.39±0.07	
E5	Ethyl decanoate	1638	1633	MS/O/RI/Std	4	6	6.32±0.00	70.19±0.01	bray, burnt, grape, nut, pear
E6	Methyl benzoate	1612	1631	MS/RI	<2	<2	0.88±0.10	0.27±0.01	
E7	Methyl dodecanoate	1804	1834	MS/O/RI/Std	6	6	3.59±0.17	13.50±0.43	coconut, fat
E8	Ethyl dodecanoate	1841	1849	MS/O/RI/Std	nd	3	nd	0.87±0.00	floral, fruit, green apple, leaf, nut
E9	Methyl hexadecanoate	2194	2243	MS/RI	<2	<2	0.029±0.00	0.01±0.00	
Lactones									
F1	Oxolan-2-one	1632	1602	MS/RI	<2	<2	0.02±0.00	0.03±0.00	
F2	5-Ethyloxolan-2-one	1694	1736	MS/O/RI/Std	5	3	1.07±0.00	0.35±0.03	coconut, coumarin, onion, sweet, warm
F3	5-Propyloxolan-2-one	1787	1796	MS/O/RI/Std	2	2	0.22±0.00	0.43±0.01	caramel, fat, nut, peach, sweet
F4	5-Butyloxolan-2-one	1910	1936	MS/O/RI/Std	4	4	4.35±0.05	1.41±0.01	coconut, fruit
F5	5-Heptyloxolan-2-one	2024	2247	MS/O/RI/Std	nd	5	nd	22.79±0.12	apricot, cocoa, coconut,

									peach, sweet
Pyrazines									
G1	2,6-dimethyl-Pyrazine	1328	1319	MS/O/RI/Std	<2	2	0.001±0.00	0.003±0.00	baked, bell pepper, green, sweet
G2	2-ethyl-6-methyl-Pyrazine	1386	1363	MS/O/RI/Std	2	<2	0.42±0.03	0.34±0.02	grass, green, nut, roasted
G3	2-ethyl-3,5-dimethyl-Pyrazine	1455	1464	MS/O/RI/Std	5	6	2200.00±196.00	2687.00±5.00	earth, must, nut, potato, roast
G4	Tetramethyl-pyrazine	1470	1457	MS/O/RI/Std	<2	2	0.05±0.00	0.03±0.00	cocoa, coffee, green, mocha, roast
Olefins									
H1	Limonene	1200	1189	MS/O/RI/Std	4	<2	0.01±0.00	0.001±0.00	citrus, orange
H2	γ-Terpinene	1246	1238	MS/RI	nd	<2	nd	1.52±0.07	
H3	Styrene	1261	1254	MS/RI/Std	<2	<2	0.01±0.00	0.15±0.00	
H4	α-Farnesene	1746	1754	MS/RI	<2	<2	-	-	
Others									
I1	2-Pentyl-furan	1231	1249	MS/RI/Std	nd	<2	nd	0.30±0.00	
I2	*p*-Cymene	1270	1261	MS/RI/Std	<2	nd	77.74±4.47	nd	
I3	Naphthalene	1746	1707	MS/RI/Std	<2	<2	0.02±0.00	0.51±0.00	

[a] Linear retention index on DB-Wax columns were determined by *n*-alkanes.

[b] Linear retention index on DB-WAX column from the literature. And "/" indicated the compound lack of reliable linear retention index value in literature.

[c] MS, identified by MS spectra; LRI, linear retention indices; O, identified by comparison of their odour description with the authentic compounds via GC–O; S, identified by comparison to standards.

[d] Sum of times detected by three assessors during DF, nd: not detected.

[e] OAV was equal to the odour concentration divided by the threshold in water. The threshold was obtained from information available in the website (https://www.vcf-online.nl) and L. J. van Gemert. "-": the threshold is not available. nd: not detected.

[f] Odour description perceived by the judges during DFA.

3.4 Identification of aroma-active compounds in pilot scale FD red jujube by OAVs

OAV analysis is frequently applied to evaluate the aroma potency based on the equilibrium between the food matrix and the air. It is generally accepted that the greater the OAV of the compound, the more important the contribution to aroma (Zhang et al., 2019). According to the principle, compounds with OAVs greater than 1 were regarded the aroma-active compounds of the samples.

There were 16 aroma-active compounds in freeze-dried red jujube identified by OAV, including alcohols (2), aldehydes (2), ketones (1), acids (1), esters (6), lactones (2), pyrazines (1) and olefin (1) (**Table 4-3**). From **Figure 4-4**, there were 12 aroma-active compounds identified by OAV joint by raw and freeze-dried red jujube, these compounds mainly contributed to the sweet and fruity notes of raw and freeze-dried red jujube. Nonanal, oct-1-en-3-ol, 5-heptyloxolan-2-one and γ-terpinene were newly identified as aroma-active compounds by OAV after FD, which would contribute the fruity, floral and sweet notes to freeze-dried red jujube. Meanwhile, butane-2,3-dione with the cream note was not detected after FD, while it had the highest OAV (OAV=2,388.62) in raw jujube. These changes of OAV might explain the differences of aroma profile between freeze-dried and raw jujube.

In further comparison with raw red jujube, pyrazines and esters contributed higher OAVs to freeze-dried red jujube (**Table 4-3**). On the other hand, 2-ethyl-3,5-dimethyl-pyrazine (OAV=2,687.00) was present the highest OAV, followed by ethyl heptanoate (OAV=479.57), and 3-hydroxybutan-2-one (OAV=233.29). This could be explained by the low odour threshold of 2-ethyl-3,5-dimethyl-pyrazine (0.00004 mg/kg) and ethyl heptanoate (0.0019 mg/kg), and the high concentration of 3-hydroxybutan-2-one (3266 μg/kg). Otherwise, ethyl decanoate (OAV=70.19), 2,3-butanediol (OAV=48.63), hexyl acetate (OAV=33.99), and 5-heptyloxolan-2-one (OAV=22.79) also had the higher OAVs in freeze-dried red jujube. In contrast, the OAVs of volatile compounds were less than 1, demonstrating that, individually, these volatiles may make only subtle contributions to the overall aroma of freeze-dried red jujube. This might be due to the high odour threshold or the low concentrations of these volatiles, such as acetic acid and oxolan-2-one with high odour threshold (99 and 20 mg/kg, respectively). In addition, oct-1-en-3-ol, 2,3-butanediol, 3-hydroxybutan-2-one, 3-methyl-butanoic acid, ethyl heptanoate, ethyl decanoate, methyl dodecanoate, 5-butyloxolan-2-one, 5-heptyloxolan-2-one and 2-

ethyl-3,5-dimethyl-pyrazine were identified as aroma-active compounds in freeze-dried red jujube by DF and OAV, demonstrating that they have the major contribution on aroma characteristics.

3.5 Identification of key aroma-active compounds in pilot scale FD red jujube by aroma recombination and omission experiments

As shown in **Figure 4-5**, the aroma profile of the complete recombinant (Model 1) was similar to that of the original sample. This result indicated the success in identification and quantification of aroma compounds of freeze-dried red jujube, because the mixtures of these odourants are very similar to those of the original samples. To further investigate the contributions of aroma-active compounds to the overall aroma profiles of freeze-dried red jujube, all compounds with OAVs ≥ 1 and DF ≥ 2 were subjected to omission experiments. As shown in **Table 4-4**, the omission models of 3-hydroxybutan-2-one, 3-oxobutan-2-yl acetate, ethyl heptanoate, methyl decanoate, ethyl decanoate, methyl dodecanoate and 2-ethyl-3,5-dimethyl-pyrazine showed very highly significant differences ($p \leq 0.001$) when compared to the complete recombinant. Additionally, when hexyl acetate and 5-propyloxolan-2-one were omitted, highly significant differences ($p \leq 0.01$) between the omission models and complete recombinant were observed. Furthermore, the significant differences ($p \leq 0.05$) were observed when 2,3-butanediol, ethyl dodecanoate, (*E*)-but-2-enoic acid, 3-methyl-butanoic acid and 5-heptyloxolan-2-one were removed. However, no significant differences were observed by the omission of the other odourants ($p > 0.05$). These results demonstrated that these 14 odourants were the key odourants in complete recombinant. Based on the results of omission tests, the aroma recombination model 2 (**Figure 4-5**) was estimated, which is consisted of above mentioned 14 odourants. Compared the model 2 with original sample, the aroma profile showed no significant difference to the original freeze-dried red jujube. This result verified these 14 compounds as the key aroma-active compounds of freeze-dried red jujube.

Table 4-4 Results of omission experiments performed on aroma reconstitutes of freeze-dried jujube

Number	Compound(s) omitted	N[a]	Significance[b]
1	**all alcohol**	7	*
1-1	Oct-1-en-3-ol	4	-

1-2	2,3-Butanediol	7	*
2	**all aldehyde**	6	-
2-1	Hexanal	5	-
2-2	Nonanal	3	-
2-3	Benzaldehyde	3	-
3	**all ketone**	10	***
3-1	3-Hydroxybutan-2-one	10	***
3-2	3-oxobutan-2-yl acetate	9	***
4	**all esters**	10	***
4-1	Methyl hexanoate	5	-
4-2	Hexyl acetate	8	**
4-3	Ethyl heptanoate	9	***
4-4	Methyl decanoate	9	***
4-5	Ethyl decanoate	10	***
4-6	Methyl benzoate	6	-
4-7	Methyl dodecanoate	10	***
4-8	Ethyl dodecanoate	7	*
5	**all acids**	10	***
5-1	3-Methyl-butanoic acid	7	*
5-2	(*E*)-But-2-enoic acid	8	**
5-3	Hexanoic acid	3	-
5-4	Heptanoic acid	5	-
5-5	Octanoic acid	2	-
6	**all lactones**	10	***
6-1	5-Ethyloxolan-2-one	3	-
6-2	5-Propyloxolan-2-one	8	**
6-3	5-Butyloxolan-2-one	2	-
6-4	5-Heptyloxolan-2-one	7	*
7	**all pyrazines**	10	***
7-1	2,6-Dimethyl-pyrazine	4	-
7-2	2-Ethyl-3,5-dimethyl-pyrazine	10	***
7-3	Tetramethyl-pyrazine	2	-
8	γ-Terpinene	6	-
9	2-Pentyl-furan	1	-
10	Naphthalene	2	-

[a]Number of correct judgments from 10 panelist evaluating the aroma difference by means of a triangle test.

[b]Significance: ***, very highly significant ($\alpha \leq 0.001$); **, highly significant ($\alpha \leq 0.01$); and *, significant ($\alpha \leq 0.05$).

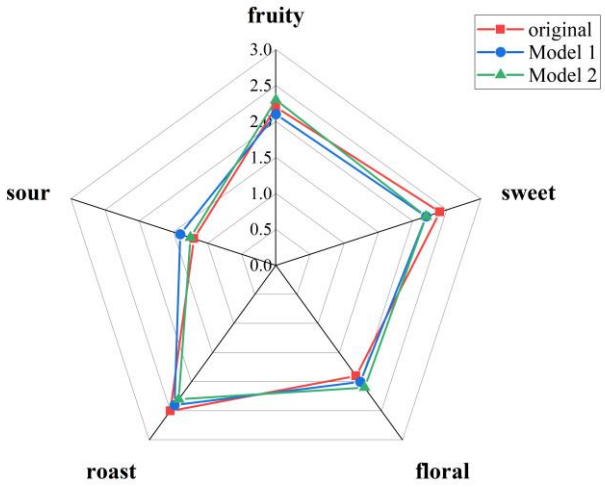


Figure 4-5 Aroma profiles of freeze-dried jujube obtained from original sample, model 1, and model 2

Compared to raw red jujube, ethyl heptanoate, hexyl acetate, 2,3-butanediol, ethyl dodecanoate and 5-heptyloxolan-2-one were new key aroma-active compounds in freeze-dried red jujube. While 3-hydroxybutan-2-one, 3-oxobutan-2-yl acetate, methyl decanoate, ethyl decanoate, methyl dodecanoate, 2-ethyl-3,5-dimethyl-pyrazine, 3-methyl-butanoic acid, (*E*)-but-2-enoic acid and 5-propyloxolan-2-one were common key aroma-active compounds of raw red jujube and freeze-dried red jujube. Overall, these composition differences of key aroma-active compounds in raw and freeze-dried red jujube caused the significant difference in their aroma profiles. For example, 2-ethyl-3,5-dimethyl-pyrazine was also key aroma-active compounds in raw jujube, but the content and OAV percentage increased after FD, the aroma profile is transformed from nut note to roast note. Moreover, hexanoic acid, hexanal and 6-methyl-5-hepten-2-one were no longer key aroma-active compounds in freeze-dried red jujube, that resulted the green and cream notes were no longer perceived and the sour note became the weakest aroma attribute in FD red jujube, these results were also consistent with the sensory evaluation results of aroma profiles in raw and freeze-dried jujube.

4. Conclusion

Forty-one volatile compounds were detected in freeze-dried red jujube, 26.71% of aroma loss was observed compared with raw red jujube. 3-Hydroxybutan-2-one, 3-oxobutan-2-yl acetate, ethyl heptanoate, methyl decanoate, ethyl decanoate, methyl dodecanoate, 2-ethyl-3,5-dimethyl-pyrazine, hexyl acetate, 3-methyl-butanoic acid, (*E*)-but-2-enoic acid, 5-propyloxolan-2-one, 2,3-butanediol, ethyl dodecanoate and 5-heptyloxolan-2-one were identified as key aroma-active compounds in freeze-dried jujube, in which ethyl heptanoate, hexyl acetate, 2,3-butanediol, ethyl dodecanoate and 5-heptyloxolan-2-one were newly identified. 2-Ethyl-3,5-dimethyl-pyrazine had the highest OAV (2,687.00) and dominated the roast note of aroma profile in freeze-dried jujube. This study will provide the technical basis for aroma retention during freeze drying in industry.

5

Chapter V. Novel insight into the evolution of volatile compounds during dynamic freeze-drying of *Ziziphus jujuba* cv. Huizao based on GC-MS combined with multivariate data analysis

The aroma had significant changes after pilot scale of freeze drying. The development of aroma in pilot scale freeze-dried red jujube is a dynamic and complex process, and chemical and enzymatic reactions will occur and result in the production of different and new aroma. The aim of this chapter was to investigate the changes of aroma, aroma precursors (sugars, fatty acid and free amino acids), and related enzyme activities of red jujube during the pilot scale freeze drying process. And to explore the correlation between aroma and aroma precursors and enzyme activities.

Gou, M., Chen, Q., Wu, X., Liu, G., Fauconnier, M.-L., & Bi, J. (2023). Novel insight into the evolution of volatile compounds during dynamic freeze-drying of Ziziphus jujuba cv. Huizao based on GC–MS combined with multivariate data analysis. Food Chemistry, 410, 135368. https://doi.org/10.1016/j.foodchem.2022.135368

Abstract: To understand the evolution of aroma in jujubes during dynamic freeze drying (FD), the relationship between aroma compounds, precursors, and related enzyme activities were analyzed. Fifty-three volatiles were identified during FD processing. After FD, the total aroma contents were increased from 11,004 to 14,603 μg/kg, ketones content was significantly decreased by 54.11%, resulted in the loss of creamy note in freeze-dried jujube (FDJ). Through the network analysis, serine, glycine, proline, valine, cysteine, arginine, glutamic acid, lysine and leucine had the significant correlation with pyrazines, dominated the roasty note of FDJ. Linoleic acid, α-linolenic acid and oleic acid with lipoxygenase had important effects on the increase of esters (from 412 to 9,486 μg/kg), contributed fruity and sweet notes of FDJ. Besides, through the Mantel test, the influence degree of factors on the formation of FDJ aroma was ranked as temperature > enzyme activity > fatty acids > amino acids.

Keywords: pilot scale freeze drying, amino acids, fatty acids, network analysis, Mantel test

1. Introduction

Red jujubes (*Zizyphus jujuba* Mill.) are both used as food and medicine in China, their unique flavour is helpful to improve consumers' attraction and enhance market competitiveness (Gou et al., 2022). With the development of freeze drying (FD), freeze-dried red jujube made from *Ziziphus jujuba* cv. Huizao, has become a popular product, with better nutrition, appearance, color and aroma. However, the causes of aroma differences between raw and freeze-dried jujube and the aroma formation pathway during FD are still unclear. The development of aroma in freeze-dried red jujube is a dynamic and complex process that depends on the combined effects of drying condition, aroma precursors and enzyme activities.

Different from lower constant freeze-drying temperature (less than 30 ^{o}C) of experimental FD machine, which usually reported in the published literature, the multi-stage and variable-temperature freeze-dried procedure was used in industry. Fortunately, same procedure could be well achieved by the pilot scale FD. In which, a higher temperature of heating plate (85~65 ^{o}C) was used to provide higher latent heat for the sublimation of water, to accelerate the FD rate and shorten the FD time. In this process, the sample temperature will gradually increase from the freezing temperature (-40 ^{o}C) to the heating plate temperature (65 ^{o}C), chemical and enzymatic reactions will occur and result in the production of different and new aroma.

In addition to higher temperature FD condition, red jujube contains rich aroma precursors, including amino acids, fatty acids and reducing sugars (J. Song et al., 2019), which could provide a variety of metabolic pathways and chemical reaction, such as Maillard reaction for the aroma formation of freeze-dried red jujube. Fatty acids are the precursors of most aliphatic alcohols, aldehydes, ketones and esters that have a variety of oxidation pathways, among which lipoxygenase (LOX) oxidation pathway is involved in the synthesis of green flavour compounds (C-6 and C-9 aldehydes and alcohols) (Boukobza et al., 2001). Reducing sugar is also a precursor for the metabolic synthesis of alcohols, acids, esters. During anaerobic respiration, monosaccharides are converted to pyruvate, which is catalyzed by dehydrogenases to form acetyl-CoA and further ester compounds (El Hadi et al., 2013; Schwab et al., 2008a). In addition, amino acids could also form esters by acetyl-CoA or form pyrazines by Maillard reaction with reducing sugar (Gonda et al., 2010).

Coupled with FD condition and aroma precursors, the aroma production of red

jujube by pilot scale FD is more complex, involving lipid oxidation, Maillard reaction and lipid-Maillard interaction. Therefore, the changes of aroma, reducing sugars, fatty acid and free amino acids, and related enzyme activities in the pilot scale freeze drying process of red jujube will be investigated; and to explore the correlation between aroma and aroma precursors and enzyme activities, main precursors of aroma-active compounds will be identified through the Mantel test and network analysis. It could provide novel insights into the aroma evolution in dynamic FD of red jujube, as well as guidance for future research including optimization of the freeze dried process to improve the aroma profile of red jujube.

2. Materials and methods

2.1. Materials and chemicals

Red jujubes (*Zizyphus jujuba* cv. Huizao) were obtained from local orchard in Akesu, Xinjiang, China, in November 2020. Mature fruits without any physical damage were selected, then collected and transported to Beijing within 2 days. All jujube samples were stored at 4 ºC controlled atmosphere storage room until used. The water content of "Huizao" was 25.57%, the pH was 5.5, and the solid soluble content was 69.0%.

Oct-1-en-3-ol, 2,3-butanediol, hexanal, (*E*)-2-hexenal, (*E*)-2-heptenal , (*E*)-2-octenal, furfural, benzaldehyde, decanal, butane-2,3-dione, 3-octanone, 3-hydroxybutan-2-one, oct-1-en-3-one, 6-methyl-5-hepten-2-one, 6,10-dimethyl-2-undecanone, acetic acid, 3-methyl-butanoic acid, pentanoic acid, (*E*)-but-2-enoic acid, hexanoic acid, heptanoic acid, (*E*)-2-hexenoic acid, octanoic acid, nonanoic acid, methyl hexanoate, ethyl hexanoate, hexyl acetate, ethyl heptanoate, methyl octanoate, ethy octanoate, methyl decanoate, ethyl decanoate, methyl dodecanoate, ethyl dodecanoate, oxolan-2-one, 5-ethyloxolan-2-one, 6-methyloxan-2-one, 5-propyloxolan-2-one, 5-butyloxolan-2-one, 5-heptyloxolan-2-one, 5-hexyloxolan-2-one, 2,6-dimethylpyrazine, 2,6-diethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, tetramethylpyrazine, limonene, γ-terpinene, naphthalene, 2-cyclohexene-1-one, *n*-alkane (C5-C40), Triton X-100, Dithiothreitol (DTT), crosslinked polyvinylpyrrolidone (PVPP), nicotinamide adenine dinucleotide (NADH), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetoacetyl coenzyme A (acetyl CoA), MES-Tris buffer (pH 6.0), Tris-HCl buffer (0.5 mol/L, pH 8.0), acetaldehyde, butanolwere purchased from Yuanye Bio-Technology (Shanghai Yuanye Bio-

Technology Co., Ltd, Shanghai, China). 3-Oxobutan-2-yl acetate, 2-ethyl-6-methylpyrazine, styrene, α-farnesene, p-cymene and MgCl2 were purchased from Macklin (Shanghai Macklin Biochemical Co., Ltd, Shanghai, China). All of the chemical standards used above with purity ≥ 99%, and other reagents were analytical grade.

2.2. Freeze drying (FD) treatment

Briefly, the kernel of jujubes was removed and the remaining part was cut into 5 mm slices; then 500 g jujube slices soaked in 80 ^{o}C water for 1 min to keep a relatively flat surface before freeze drying. Jujube slices were drained in a colander and put into -40 ^{o}C refrigerator for 48 h. A pilot scale freeze dryer (Advantech Co., Ltd. China) was used with drying conditions was as follows: The cold trap temperature and vacuum pressure were -30 ^{o}C and 60 Pa, respectively; the drying temperature of the heating plate was from room temperature (25 ^{o}C) to 85 ^{o}C within 45 min and kept for 3 h, then decreased to 70 ^{o}C within 30 min and maintained for 5 h, and finally decrease to 65 ^{o}C within 30 min and kept for 1 h. The sample tray was in the middle of two heating plates and not directly connected, the diagram was shown in **Figure 4-1**. At the same time, the temperature of jujube slices was monitored online through the temperature probe which equipped in FD machine (**Figure 4-2**). The dynamic FD process included 0-10 stages, with drying time of 0, 105, 165, 225, 285, 345, 405, 465, 525, 585 and 645 min, respectively.

2.3 Volatile compounds analysis

2.3.1 Extraction of volatile compounds by using Headspace solid-phase microextraction (HS-SPME)

The extraction method by HS-SPME of red jujube aroma was described by Gou et al., (2022).

2.3.2 Determination of volatile compounds using gas chromatography–mass spectrometry (GC–MS)

The volatile compounds were identified by GC–MS (QP-2010, Shimadzu, Japan) equipped with a DB-Wax column (60 m × 0.25 mm, 0.25 μm). The temperature programs were according to (Gou et al., 2022). The aroma compounds were identified by comparing the NIST17 library of the GC-MS and were confirmed by the retention indices (RI) and authentic aroma standards. The RI was calculated on the basis of the linear retention times of the *n*-alkanes (C5−C40) in the DB-WAX

columns under the same GC-MS conditions. Internal standard method was used for aroma quantitative analysis (2 μL 2-cyclohexene-1-one, 1mg/L). The content of each volatile compound was calculated based on the GC peak areas related to that of internal standard.

2.3.3. Odour activity value (OAV)

$$OAV=C/OT \quad (1)$$

where C was the concentration of the compound and OT was its orthonasal detection odour threshold. The threshold values referred to the literature in water (Gou et al., 2022).

2.4. Sensory evaluation

The panelist selection and training methods were according to Gou et al., (2022) and Pu et al., (2020). The sensory evaluation was performed by 10 panelists (4 males and 6 females aged 23-28, healthy, without rhinitis, and nonsmokers) who were experienced and engaged in food flavour research for sensory evaluation. Panelists were trained for 4 weeks: Firstly, they were trained to distinguish and describe the aroma standards of red jujube for 4 weeks. Secondly, the panelists proceeded to conduct sensory evaluation of the red jujube sample. The aroma descriptors of red jujube were determined according to the experts' discussion on sensory attributes. In this study, The descriptors of red jujube were creamy (3-hydroxybutan-2-one), floral (5-butyloxolan-2-one), green (hexanal), fruity (methyl dodecanoate), roasty (2,6-dimethylpyrazine), sweet ((*E*)-but-2-enoic acid), sour (acetic acid), rancid (3-methyl-butanoic acid) and nut (2-ethyl-6-methyl-pyrazine) (Pu et al., 2022). Finally, the quantitative descriptive analysis (QDA) was conducted in triplicate by panelists, and scores for each sample in 0.5 increments, from 0.0 to 3.0 on the basis of 7-point scales (0, none; 1.5, moderate; and 3, very strong).

2.5. Aroma precursor analysis

2.5.1 Sugar compounds analysis

Sucrose, glucose, and fructose in red jujube were analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (ICS-3000, DIONEX Co., Ltd. China) according to the method of Song et al., (2019).

2.5.2 Free amino acids analysis

Amino acid was analyzed by automatic amino acid analyzer (L-8900, Hitachi, Japan) according to the method of Song et al. (2019).

2.5.3 Fatty acids analysis

The fatty acids of jujube were detected by gas chromatograph (GC) equipped with a flame ionization detector (FID) detector (GC, 2010, Shimadzu, Japan) according to the national standard of China (GB 5009.168-2016). Firstly, the sample is hydrolyzed and the fatty acids are extracted with an ether solution, and then saponified and methyl esterified under alkaline conditions to generate fatty acid methyl esters, which are analyzed by capillary column gas chromatography and the content of fatty acid methyl esters is quantitatively determined by the internal standard method. Analysis was performed with an initial column temperature of 130 °C for 1 min and then increased to 170 °C at a rate of 6.5 °C/min, continuously increased to 215 °C at a rate of 2.75 °C/min and held for 12 min, then increased to 230 °C at a rate of 4 °C/min and held for 3 min. The split ratio of the carrier gas (helium) was 50:1. The injection volume was 1.0 μL. Identification and quantification of different fatty acids was conducted by internal standard method and expressed in mg/100 g dry basis. C11:0 was used as the internal standard.

2.6. Analysis of enzymes activities

2.6.1 Lipoxygenase (LOX) activity

Sodium phosphate buffer (0.50 mol/L, pH 6.5) and 0.5% Triton X-100 was used for LOX extraction (Lyu et al., 2021). And LOX activity was assayed according to Amanpour et al. (2019).

2.6.2 Alcohol dehydrogenase (ADH) activity

The MES-Tris buffer (0.1 mol/L, pH 6.0) with DTT (2 mmol/L) and PVPP (1%) was used for ADH extraction. ADH activity assayed according to Zhou et al. (2019) and Lara et al., (2003) with slight modifications. The reaction system containing 0.3 mL ADH crude enzyme, 2.4 mL MES-Tris buffer (pH 6.0), 0.15 mL NADH (1.6 mmol/L) and 0.15 mL acetaldehyde (80 mmol/L). The above reaction substrate was mixed and determined at 340 nm for 1 min.

2.6.3 Alcohol acyltransferase (AAT) activity

The Tris-HCl (0.5 mol/L, pH 8.0), containing 0.1%（V/V）Triton X-100 and 0.3 mg/g PVPP was used for AAT extraction. For AAT activity, the reaction substrate was 2.5 mL Tris-HCl (0.5 mol/L, pH 8.0, containing 0.5 mmol/L $MgCl_2$), 150 μL acetyl CoA (0.5 mol/L, pH 8.0, containing 0.5 mmol/L acetyl CoA), 50 μL butanol (0.5 mol/L, pH 8.0, containing 20 mmol/L acetyl CoA) and 150 μL AAT crude enzyme. The above reaction substrate was incubated at 35 °C in water bath for 15

min, then 100 μL 1 mmol/L DTNB was added and allowed to stand at room temperature for 10 min and determined at 412 nm for 1 min (D. Zhou et al., 2019).

One unit (U) represents the variation of absorbance per minute. The specific activity of all enzymes was defined as U/g.

2.7. Statistical Analysis

Software of SPSS version 20.0 (SPSS Inc., Chicago, IL) was applied for statistical treatment. Duncan's multiple test was used to verify significant differences among the samples at $p < 0.05$ level. Contents of different components were presented as the mean ± SD (standard deviation). The clustered heatmap was plotted using TBtools version 1.0686 (Heatmap Illustrator, China). The Mantel test analysis was performed using the OmicStudio tools at https://www.omicstudio.cn/tool. The network correlation analysis was constructed using Cytoscape (v.3.4.0).

3. Results and discussion

3.1. Sensory analysis of red jujube during freeze drying

Aroma profiles of red jujube at different FD stages were evaluated by trained panelists. As shown in **Figure 5-1**, the aroma profiles could be divided to two groups, the stages 0 (raw)-3 was one group with sweet (2.7), fruity (2.5), creamy (2.4), nut (1.8), sour/rancid (1.5), floral (1.4) and green (1.1) notes. While the stages 4-10 was the other group with roasty (2.7), sweet (2.4), fruity (2.2), floral (1.4) and sour/rancid (0.8) notes. Among these aroma contributors, the aroma intensity of creamy, sour/rancid, green, and nut decreased significantly with FD time increased, and performed the lowest at the end of FD (stage 10), especially for creamy, green, and nut notes. In addition, the aroma intensity of roasty increased with FD time increased from stage 4. The aroma profile transformed from sweet dominated to roasty dominated during FD processing. To further investigate the aroma differences among samples, GC-MS combined with OAV were applied to analyze the volatile compounds responsible for the aroma differences of samples.

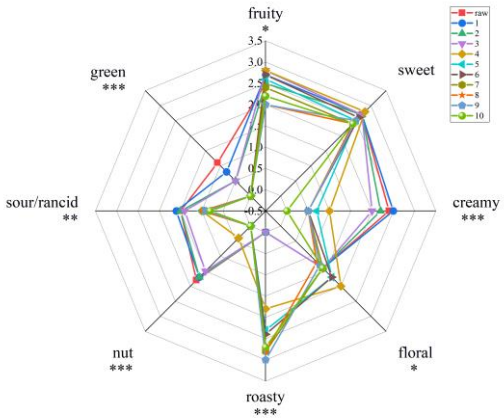
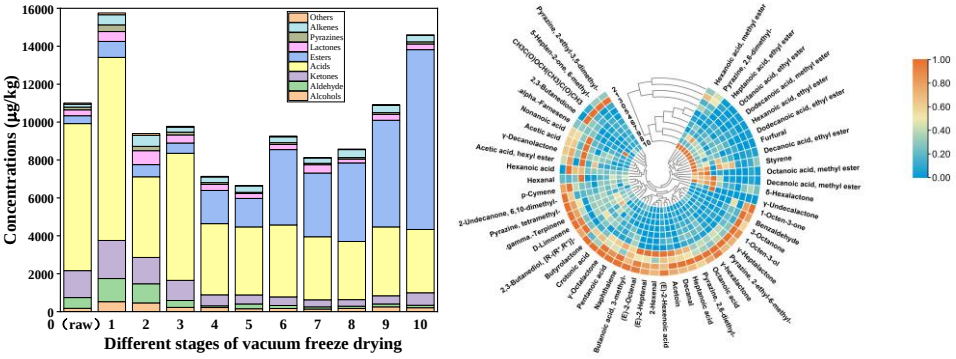


Figure 5-1 The changes in aroma profiles in red jujube during different freeze drying stages (***, very highly significant ($p \leq 0.001$); **, highly significant ($p \leq 0.01$); and *, significant ($p \leq 0.05$)

3.2. Dynamic changes in aroma compounds of red jujube during freeze drying

A total of 53 aroma compounds were detected in the red jujube during FD processing for stage 0-10 (0-645 min), including 2 alcohols, 7 aldehydes, 7 ketones, 9 acids, 10 esters, 7 lactones, 5 pyrazines, 4 alkenes and 2 others **(Table 5-1)**. As shown in **Table 5-1 and Figure 5-2(a)**, the content of total aroma compounds in red jujube was significantly increased from 11,005 μg/kg to 14,605 μg/kg at stage 0-10. As reported, the average loss ratio of volatile compounds of freeze-dried banana slices and carvone in bread crumbs was 37.5% and 55%, respectively (Dimelow et al., 2005; Mui et al., 2002). It can be seen, pilot scale freeze drying could enhance the aroma compared with traditional constant FD. However, the concentrations of aroma compounds undergoes complex changes during pilot scale FD processing, where they increased from 11,005 μg/kg to 15,726 μg/kg (stage 0 to 1), then decreased to 6,657 μg/kg (stage 5), and finally increased to 14,605 μg/kg (stage 10). The contents of ketones, aldehydes and acids showed an obviously decreased trend during FD; meanwhile, esters showed an increased trend with FD time increased **(Figure 5-2(a))**. The clustering content heatmap revealed that there were two obvious groups (stage 0-3 and stage 4-10) **(Figure 5-2(b))**, which was in accordance with the sensory evaluation result. These changes of aroma composition could explain the transformation of aroma profile in red jujube after FD.



(a) (b)

Figure 5-2 The contents of different group of aroma compounds (a) and clustering aroma compounds content heatmap (b) in red jujube during different stage of freeze drying.

Table 5-1 The contents of aroma compounds in red jujube at different stage of freeze drying**.**

	Compounds (μg/kg)	LRI([a]/[b])	Freeze drying stage										
			raw	1	2	3	4	5	6	7	8	9	10
A1	Oct-1-en-3-ol	1450/1448	138±21bcd	346±29a	313±7a	153±4b	101±6cde	89.51±12.81de	105±8bcde	79.79±1.24e	82.98±1.66e	117±6bcde	141±10bc
A2	2,3-Butanediol	1556/1553	39.28±9.74e	178±19a	147±4ab	74.59±3.67de	115±7bc	64.11±2.92de	60.26±17.52de	59.22±4.07de	89.00±0.23cd	136±0b	66.28±5.78de
	Total		177	524	460	228	216	154	165	139	172	253	207
B1	Hexanal	1083/1078	96.46±1.03a	34.12±0.35cd	37.54±4.70cd	40.92±1.30cd	23.56±1.69ef	21.08±3.48f	33.30±0.12de	37.17±0.83cd	44.15±3.35c	54.98±0.83b	56.51±4.45b
B2	(*E*)-2-Hexenal	1216/1228	35.43±1.81a	28.13±0.37b	24.96±1.12c	11.26±0.16d	nd	nd	nd	nd	nd	nd	nd
B3	(*E*)-2-Heptenal	1323/1318	25.16±0.39a	20.32±0.12b	20.42±0.44b	12.25±1.68c	nd	nd	nd	nd	nd	nd	nd
B4	(*E*)-2-Octenal	1429/1410	81.25±3.37a	76.34±5.45a	77.89±0.95a	41.15±16.28b	nd	nd	nd	nd	nd	nd	nd
B5	Furfural	1460/1479	1.80±0.01d	nd	nd	nd	nd	1.11±0.13e	3.23±0.14c	4.21±0.12b	4.36±0.33b	1.4±0.37de	14.04±0.01a
B6	Benzaldehyde	1480/1508	294±5c	1025±85a	823±43b	233±10cd	67.42±11.34e	231±0cd	125±28de	70.75±0.69e	67.84±0.68e	99.67±16.42e	63.41±17.94e
B7	Decanal	1520/1498	29.72±5.20b	39.48±1.45a	27.73±0.62bc	22.33±1.26c	nd	nd	nd	nd	nd	nd	nd
	Total		564	1223	1012	361	90.98	253	162	112	116	156	134
C1	Butane-2,3-dione	997/979	83.73±1.12b	356±40a	nd	108±2b	nd	nd	nd	nd	nd	nd	nd
C2	3-Octanone	1253/1240	18.62±1.25c	67.75±6.20a	41.47±1.77b	13.63±1.47cd	8.75±0.96d	7.00±0.83d	5.45±0.45d	6.66±0.75d	7.35±0.22d	8.43±0.34d	10.89±0.01cd
C3	3-Hydroxybutan-2-one	1284/1286	1188±93ab	1258±57a	1019±62bc	844±18c	461±11d	346±35de	305±52de	254±16e	263±20e	267±26e	471±30d
C4	Oct-1-en-3-one	1290/1296	20.84±0.91c	29.89±0.74b	37.96±4.28a	nd	nd	nd	nd	nd	nd	nd	nd
C5	6-Methyl-5-hepten-2-one	1338/1342	28.17±6.20c	105±16a	49.80±1.71b	23.69±0.98c	34.53±0.01bc	35.32±0.02bc	32.08±0.01bc	29.98±0.01bc	29.85±0.01bc	36.43±0.01bc	36.31±0.01bc
C6	3-Oxobutan-2-yl acetate	1378/-	15.09±1.46c	38.60±1.15a	13.77±0.92c	15.13±0.54c	12.17±0.14c	12.10±0.49c	14.38±2.41c	10.61±0.80c	12.38±0.51c	11.89±1.45c	20.77±2.49b
C7	6,10-Dimethyl-2-undecanone	1450/1660	67.86±19.73cde	152±8b	225±26a	58.37±17.52de	62.87±2.16de	76.87±2.81cde	94.07±1.07cd	73.71±4.17cde	31.60±5.30e	104±13bcd	114±9bc

	Total		1422	2007	1387	1063	579	477	451	375	344	428	653
D1	Acetic acid	1449/1429	1352±186a	1052±0ab	1041±40ab	687±144bc	535±20c	754±20bc	783±143bc	836±53bc	602±44c	697±87bc	585±86c
D2	3-Methylbutanoic acid	1666/1680	492±5b	565±0b	799±12a	560±10b	284±35cd	316±46c	90.02±7.96e	50.51±2.70e	74.71±9.00e	50.96±0.01e	213±8d
D3	Pentanoic acid	1733/1762	224±19b	273±0a	303±8a	192±20b	117±1c	112±6c	125±3c	108±21c	105±5c	98.88±14.38c	112±2c
D4	(*E*)-But-2-enoic acid	1745/1750	29.87±0.33bc	34.52±9.08bc	72.34±15.00a	54.1±16.8ab	33.5±13.78bc	16.26±1.20c	31.95±6.52bc	15.21±3.40c	19.55±1.19bc	30.66±4.39bc	22.68±2.31bc
D5	Hexanoic acid	1846/1849	4270±146b	5941±161a	510±75d	4181±676b	2098±50c	1845±147c	2214±90c	1756±7c	1760±119c	2220±420c	1992±466c
D6	Heptanoic acid	1950/1943	502±59b	639±3a	497±1b	410±5c	277±1d	209±17de	245±6d	221±1de	209±7de	217±2de	163±18e
D7	(*E*)-2-Hexenoic acid	1967/1994	156±4a	116±17a	152±13a	115±10a	52.76±27.46b	34.37±5.83b	40.57±6.93b	44.62±1.21b	48.09±0.91b	45.30±13.70b	41.08±5.72b
D8	Octanoic acid	2060/2086	470±23b	894±1a	726±111a	424±14bc	325±15bcd	249±13d	265±16cd	248±3d	230±75d	238±25d	181±8d
D9	Nonanoic acid	2178/2171	261±57a	143±31b	152±12b	78.1±7.25bc	36.09±1.52c	50.47±7.61c	nd	45.29±0.99c	22.42±0.58c	34.91±0.001c	32.18±0.01c
	Total		7757	9658	4252	6701	3758	3586	3795	3325	3070	3633	3342
E1	Methyl hexanoate	1184/1177	60.73±2.37c	101±0b	53.16±2.58cd	33.81±2.06cde	22.93±1.25de	15.12±4.08e	30.99±11.81cde	14.06±2.12e	34.00±1.93cde	42.53±16.38cde	142±22a
E2	Ethyl hexanoate	1245/1241	61.59±2.33cd	19.3±0.76d	27.61±3.91d	60.04±2.32cd	97.37±3.38bcd	54.14±1.42d	93.03±7.76bcd	26.99±1.26d	142±2bc	176±65b	417±28a
E3	Hexyl acetate	1272/1265	20.88±1.72a	17.90±0.19ab	11.43±0.07c	4.47±0.19e	15.26±1.45b	6.68±0.89de	6.66±0.14de	5.74±0.43de	5.68±0.47de	6.66±0.36de	8.18±0.14d
E4	Ethyl heptanoate	1326/1342	6.06±0.96d	35.04±0.24cd	8.84±0.01d	7.29±0.01d	9.14±0.02d	24.10±0.01cd	33.75±0.01cd	41.85±1.41cd	67.98±9.85c	276±28b	350±32a
E5	Methyl octanoate	1372/1302	15.67±0.70e	43.05±0.79d	24.14±0.51de	15.65±7.54e	18.38±0.03e	23.8±1.66de	42.35±11.12d	19.77±4.10e	265±0b	296±0a	230±10c
E6	Ethyl octanoate	1457/1441	nd	nd	nd	36.84±0.02h	66.13±0.01g	85.54±0.01f	117±0d	96.37±0.01e	195±0c	253±0 b	672±0a
E7	Methyl decanoate	1593/1636	121±15f	276±7e	164±4f	136±3f	164±4f	212±66ef	979±7d	2155±52a	1494±0b	1566±1b	1292±37c
E8	Ethyl decanoate	1638/1633	35.08±5.80f	220±14ef	147±33ef	39.34±0.43f	576±29cd	381±115de	1517±30b	413±138de	806±6c	1205±22b	2228±180a
E9	Methyl dodecanoate	1804/1834	90.87±4.64d	127±0d	124±0d	84.09±9.83d	84.09±9.83d	283±65cd	529±112bc	275±17cd	541±40bc	682±245b	1644±56a
E10	Ethyl dodecanoate	1841/1849	nd	nd	84.20±10.50def	27.47±1.76ef	698±29bc	418±3cdef	626±6bcd	315±48cdef	593±118bcde	1124±361b	2503±282a
	Total		412	839	644	445	1751	1503	3974	3410	4144	5627	9486
F1	Oxolan-2-one	1632/1602	73.54±4.93de	94.88±14.21cde	318±12a	210±9b	144±0bc	116±16cd	87.98±38.88cde	151±26bc	40.41±11.50e	131±8cd	120±12cd

F2	5-Ethyloxolan-2-one	1694/1736	118±15b	222±10a	215±4a	106±1bc	102±2bcd	69.06±1.96de	102±0bcd	94.74±4.51bcde	61.99±1.84e	76.66±18.18cde	68.96±11.15de
F3	6-Methyloxan-2-one	1773/1751	14.07±2.89f	25.85±0.21d	21.56±0.42de	22.38±0.36de	18.72±1.20ef	18.78±1.15ef	25.31±1.72d	43.86±2.15ab	35.94±2.11c	39.64±0.71bc	45.99±0.98a
F4	5-Propyloxolan-2-one	1787/1796	26.38±0.40c	50.33±1.11a	39.87±1.97b	21.89±1.31cd	11.39±0.53e	11.25±0.53e	12.80±2.05e	10.73±1.29e	10.99±1.33e	13.59±1.52e	20.16±0.36d
F5	5-Butyloxolan-2-one	1910/1936	58.00±2.38b	83.00±0.49a	83.30±5.90a	51.89±0.71b	35.00±0.34cde	31.19±1.73de	32.87±0.67cde	41.37±1.91c	26.18±2.06e	36.83±2.30cd	32.68±3.24cde
F6	5-Heptyloxolan-2-one	2024/2247	nd	nd	nd	nd	nd	nd	nd	88.41±0.01a	23.92±0.20b	8.01±0.10c	4.58±1.55d
F7	5-Hexyloxolan-2-one	2152/2113	24.57±0.38a	19.74±2.63ab	17.54±2.75bc	10.57±0.57def	12.44±0.54cd	10.90±1.00de	10.79±0.59de	9.93±2.37def	4.96±0.28f	5.47±0.28ef	5.73±0.25ef
	Total		315	496	695	423	324	257	272	440	204	311	298
G1	2,6-Dimethylpyrazine	1328/1319	11.87±2.00g	20.24±0.20c	18.73±0.06d	11.62±1.58h	6.06±0.12k	7.75±3.76j	14.36±0.01e	10.85±0.01i	11.87±8.35f	22.62±1.77b	34.49±0.13a
G2	2-Ethyl-6-methylpyrazine	1386/1363	85.52±1.54c	165±6a	123±8b	78.51±2.59c	33.03±0.48def	27.03±2.11ef	31.80±4.59ef	22.94±3.05f	33.00±0.50def	38.07±2.35de	47.06±2.50d
G3	2,6-Diethylpyrazine	1430/1440	22.51±1.28d	69.16±2.39a	40.77±0.81b	27.69±0.92c	18.39±0.01e	8.45±0.36f	11.66±0.43f	9.65±0.36f	nd	nd	nd
G4	2-Ethyl-3,5-dimethylpyrazine	1455/1464	14.96±3.09c	81.7±9.27a	35.55±1.81b	22.96±0.12c	20.33±0.81c	14.96±0.45c	26.25±0.01bc	22.54±0.01c	21.06±0.01c	20.31±0.48c	22.80±0.21c
G5	Tetramethylpyrazine	1470/1457	8.70±0.22j	13.11±0.32b	14.28±1.61a	11.51±0.27c	10.28±0.29f	10.41±0.71e	10.67±1.52d	9.73±0.47h	10.11±0.84g	9.28±0.33i	8.56±0.01k
	Total		144	349	232	152	88	69	95	76	76	90	113
H1	Limonene	1200/1189	90.2±3.18d	449±3a	481±1a	190±20c	216±14bc	229±11bc	201±2c	236±17bc	226±18bc	271±31b	208±0c
H2	*γ*.-Terpinene	1246/1238	nd	18.82±0b	23.71±2.56a	9.51±2.29cd	8.28±0.26cd	7.37±0.52cd	7.09±0.1d	7.68±0.42cd	6.01±0.09d	11.49±0.56c	7.03±0.06d
H3	Styrene	1261/1254	14.20±1.7gh	18.64±0.99g	34.28±0.37f	11.86±1.17gh	56.63±3.94e	65.95±0.21d	73.49±1.60bc	8.85±1.53h	68.17±0.36cd	78.47±0.70b	91.95±3.23a
H4	*α*-Farnesene	1746/1754	45.33±8.07b	48.70±1.31b	44.18±1.80b	43.81±0.80b	16.38±1.90c	26.11±1.79c	23.90±0.23c	23.31±4.69c	120±0a	21.70±1.23c	26.79±0.55c
	Total		150	535	583	255	297	328	305	276	420	383	334
I1	*p*-Cymene	1270/1261	10.09±1.23b	29.67±4.56a	23.97±0.35a	14.19±1.04b	3.60±0.20c	9.95±0.52b	11.57±0.49b	10.93±1.30b	8.56±1.39bc	12.68±0.05b	12.69±0.22b
I2	Naphthalene	1746/1707	53.54±2.54b	65.75±6.98a	66.11±0.12a	30.60±3.09c	27.67±1.89cd	19.59±0.55de	27.67±1.89cd	15.36±1.28e	20.76±1.56cde	19.49±0.24de	24.83±0.01cde
	Total		63.63	95.42	90.08	44.79	31.27	29.54	39.24	26.30	29.32	32.16	37.53

Mean values with different lower-case letters in the same row correspond to significant differences at $p < 0.05$. Data are represented as the mean ± SD;

"nd": Not detected. [a]LRI: Retention indices on DB-Wax columns were determined by *n*-alkanes.

[b]LRI: Retention indices on DB-Wax columns from literature. And "-" indicated the compound lack of reliable linear retention index value in literature.

Furthermore, odour activity value (OAV) was applied to explain the contribution of compounds to the overall aroma profile **(Table 5-2)**. Thirty aroma-active compounds (OAV > 1) were identified in all freeze-dried samples including 2 alcohols, 4 aldehydes, 5 ketones, 2 acids, 8 esters, 3 lactones, 3 pyrazines, 1 alkenes and 2 others. Stage 0-3 contained 27 important aroma compounds (OAV > 1), in which, ketones, aldehydes, alcohols, esters and pyrazines contributed the higher OAVs, and presented "creamy, fruit, sweet, green, floral and nut" characters. However, in the stage 4-10, there were only 20 aroma-active compounds (OAV > 1) in freeze dried samples, in which, esters and pyrazines dominated the OAVs, they contributed the sweet, fruit and roasty notes, respectively. These results were consisted with above discussed sensory evaluation.

Acids were the most numerous class of volatile compounds detected in the raw red jujube. The most abundant acids were acetic acid and hexanoic acid, with sour note. But they did not contribute a strong sour profile to red jujube due to the higher threshold. Otherwise, hexanoic acid, 3-methyl-butanoic acid and (*E*)-but-2-enoic acid were identified the key aroma-active compounds in "Huizao", they contributed the "sour and sweet" notes to red jujube (Gou et al., 2022). However, the contents of acids decreased significantly during FD processing and no longer provide the sour characteristics of freeze-dried jujube. In Song et al., (2020) study, red jujube had the highest content of total acids after constant lower temperature FD (25 °C). The different result might be due to the totally different drying condition. The multi-stage and higher temperature used in this study could promote the chemical reaction occurred, and leading the acids transformed to esters. In addition, acids might be discharged by vacuum pump, due to they have lower vapor pressure and easily vaporized. Acetic acid also had an obvious loss ratio (45.90%), which might be due to its polarity and better water solubility and easier evaporation with water, or involved in the chemical reaction.

Table 5-2 The odor activity value (OAV) of aroma compounds in red jujube at different stages of freeze drying.

	Compounds	Freeze drying stage										
		raw	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A1	Oct-1-en-3-ol	92	231	209	102	67	60	70	53	55	78	94
A2	2,3-Butanediol	<1	2	2	<1	1	<1	<1	<1	<1	1	<1
B1	Hexanal	19	7	8	8	5	4	7	7	9	11	11
B2	(*E*)-2-Hexenal	<1	<1	<1	<1	nd	nd	nd	nd	nd	nd	nd
B3	(*E*)-2-Heptenal	2	2	2	<1	nd	nd	nd	nd	nd	nd	nd
B4	(*E*)-2-Octenal	27	25	26	14	nd	nd	nd	nd	nd	nd	nd
B5	Furfural	<1	nd	nd	nd	nd	<1	<1	<1	<1	<1	<1
B6	Benzaldehyde	<1	1	1	<1	<1	<1	<1	<1	<1	<1	<1
B7	Decanal	10	13	9	7	nd	nd	nd	nd	nd	nd	nd
C1	Butane-2,3-dione	84	356	nd	108	nd	nd	<1	nd	nd	nd	nd
C2	3-Octanone	<1	3	2	<1	<1	<1	<1	<1	<1	<1	<1
C3	3-Hydroxybutan-2-one	85	90	73	60	33	25	22	18	19	19	34
C4	Oct-1-en-3-one	6946	9962	12653	nd	nd	nd	nd	nd	nd	nd	nd
C5	6-Methyl-5-hepten-2-one	<1	2	<1	<1	<1	<1	<1	<1	<1	<1	<1
C6	3-Oxobutan-2-yl acetate	/	/	/	/	/	/	/	/	/	/	/
C7	6,10-Dimethyl-2-undecanone	/	/	/	/	/	/	/	/	/	/	/
D1	Acetic acid	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
D2	Butanoic acid, 3-methyl-	<1	1	2	1	<1	<1	<1	<1	<1	<1	<1
D3	Pentanoic acid	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
D4	(*E*)-But-2-enoic acid	/	/	/	/	/	/	/	/	/	/	/
D5	Hexanoic acid	5	7	<1	5	2	2	2	2	2	2	2
D6	Heptanoic acid	1	1	<1	<1	<1	<1	<1	<1	<1	<1	<1
D7	(*E*)-2-Hexenoic acid	/	/	/	/	/	/	/	/	/	/	/
D8	Octanoic acid	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
D9	Nonanoic acid	<1	<1	<1	<1	<1	<1	nd	<1	<1	<1	<1
E1	Methyl hexanoate	<1	1	<1	<1	<1	<1	<1	<1	<1	<1	2
E2	Ethyl hexanoate	12	4	6	12	19	11	19	5	28	35	83

E3	Hexyl acetate	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	0
E4	Ethyl heptanoate	3	18	5	4	5	13	18	22	36	145	184
E5	Methyl octanoate	<1	<1	<1	<1	<1	<1	<1	<1	1	1	1
E6	Ethy octanoate	0	0	0	2	3	4	6	5	10	13	35
E7	Methyl decanoate	28	64	38	32	38	49	228	501	348	364	301
E8	Ethyl decanoate	7	44	29	28	115	76	303	83	161	241	446
E9	Methyl dodecanoate	61	84	83	56	56	189	352	183	361	455	1096
E10	Ethyl dodecanoate	nd	nd	<1	<1	2	1	2	<1	1	3	6
F1	Oxolan-2-one	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
F2	5-Ethyloxolan-2-one	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
F3	6-Methyloxan-2-one	/	/	/	/	/	/	/	/	/	/	/
F4	5-Propyloxolan-2-one	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
F5	5-Butyloxolan-2-one	<1	13	13	8	5	5	5	6	4	6	5
F6	5-Heptyloxolan-2-one	nd	nd	nd	nd	nd	nd	nd	42	11	4	2
F7	5-Hexyloxolan-2-one	22	18	16	10	11	10	10	9	5	5	5
G1	2,6-Dimethylpyrazine	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
G2	2-Ethyl-6-methylpyrazine	2	4	3	2	<1	<1	<1	<1	<1	<1	1
G3	2,6-Diethylpyrazine	4	12	7	5	3	1	2	2	nd	nd	nd
G4	2-Ethyl-3,5-dimethylpyrazine	374	2043	889	574	508	374	656	563	527	508	570
G5	Tetramethylpyrazine	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
H1	Limonene	3	13	14	6	6	7	6	7	7	8	6
H2	γ-Terpinene	nd	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
H3	Styrene	<1	<1	<1	<1	<1	1	1	<1	1	1	1
H4	α-Farnesene	/	/	/	/	/	/	/	/	/	/	/
I1	*p*-Cymene	2	6	5	3	<1	2	2	2	2	3	3
I2	Naphthalene	9	11	11	5	5	3	5	3	3	3	4

As the second most abundant class of volatile compound in raw red jujube, ketones also contributed the most OAVs to the overall aroma of raw red jujube. A total of 7 ketones were detected throughout the FD stage, and were mainly existed in the front period of FD (stage 0-3). Among these ketones, butane-2,3-dione, 3-hydroxybutan-2-one and oct-1-en-3-one might contribute the creamy, fruit and green aroma to red jujube because of their relatively low thresholds. Furthermore, butane-2,3-dione, 3-hydroxybutan-2-one, 6-methyl-5-hepten-2-one and 3-oxobutan-2-yl acetate were identified the key aroma-active compounds in "Huizao" (Gou et al., 2022). Similar to acids, most of ketones also decreased obviously with FD time, except for 6,10-dimethyl-2-undecanone, which might be produced by amino acid degradation or unsaturated fatty acid oxidation (Zhang et al., 2019).

Esters provided the fruity, sweet, and floral notes for red jujube. As shown in **Table 5-1**, as the FD time increased, the numbers and content of esters increased, especially ethyl esters, which enhance the fruity and sweet note of red jujube. However, in traditional constant FD, esters showed a decreased trendy (Chin et al., 2008). In our previous study, only methyl decanoate, ethyl decanoate and methyl dodecanoate were identified as the key aroma-active compounds, meanwhile, ethyl heptanoate, ethyl dodecanoate and hexyl acetate also been key aroma-active compounds in "Huizao" after FD. Otherwise, esters also contributed the major OAVs during later FD stage (4-10) (**Table 5-2**).

Pyrazines are generally results from the Maillard reaction, which are more favorable at high temperature. Though the percentage of pyrazines was decreased from 1.30% to 0.77% after FD, the OAVs contribution become higher (from 4.87% to 19.75%) (**Table 5-1**). They have an important contribution to the nut flavour of raw red jujube and roasty flavour of freeze-dried red jujube due to the low threshold. And the 2-methypyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine and 2-ethyl-3,5-dimethyl-pyrazine were identified as the key aroma compound in raw red jujube (Gou et al., 2022; Jiancai Zhu & Xiao, 2018a).

A total 7 aldehydes were identified at different stages of FD, some aldehydes were not detected after stage 3, such as (*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2-octenal and decanal. Aldehydes have green, fatty, grassy, and fresh characteristics (Gou et al., 2021). Thus, from stage 4, the red jujube samples no longer perceived the green note (**Figure 5-1**). However, the content of furfural increased with FD time, with sweet, caramel, nutty, and baked notes. In general, furfural was commonly produced

through non-enzymic browning, which could be promoted by the higher FD temperature. Alcohols were another important class of compounds for red jujube samples. The content of alcohols presented a slightly increase during FD, as was observed with Song et al. (2020). That might be caused by glucose metabolism, amino acid decarboxylation and dehydrogenation with prolonged FD time or oxidation and degradation of polyunsaturated fatty acids (Ye et al., 2022). Among the detected alcohols, oct-1-en-3-ol was key aroma compound in raw red jujube, and 2,3-butanediol was the key aroma compound in freeze-dried "Huizao" (Jiancai Zhu & Xiao, 2018a). Lactones might be generated from the β-oxidation of fatty acids (Xi et al., 2012b). The lactones showed maximum contents at the stage 3, and then decreased, but the contents had no significant changes after FD **(Table 5-1)**. Among these lactones, 5-propyloxolan-2-one, 5-butyloxolan-2-one and 5-ethyloxolan-2-one with sweet and fruity notes were key aroma compounds in raw "Huizao", and 5-heptyloxolan-2-one appeared from stage 7, was also identified as the key aroma compound of freeze-dried "Huizao".

3.3 Changes in aroma precursors in red jujube during freeze drying

3.3.1 Changes in contents of sugars during freeze drying

Sugars not only enhance the interaction between sweet and aroma compounds but also are the main precursor of aroma (Saint-Eve et al., 2014). **Table 5-3** presents the sugar contents calculated on a dry basis in red jujube samples, revealed that raw samples had the highest content (759 mg/g) of total sugars. Glucose had the highest loss ratio (43.89%), followed by fructose (28.78%) and sucrose (26.88%) after FD. It is illustrated the main components involved in the reaction were reducing sugars, especially glucose. Reducing sugars could form esters under the action of enzymes, and can also undergo Maillard reaction with amino acids at high temperatures to generate pyrazines (J. Song et al., 2019).

Table 5-3 The contents of sugars, free amino acids and fatty acids (of dry weight basis) in red jujube at different freeze drying stages.

Types	Compositions	Freeze drying stage										
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sugars (mg/g)	Glucose	218±13a	169±5abc	207±14ab	168±28abc	91.93±20.89c	155±35abc	116±43bc	177±36abc	129±2abc	124±19abc	123±18abc
	Sucrose	334±5ab	247±57ab	319±22ab	236±43ab	169±36b	265±63ab	210±76ab	311±62ab	272±8ab	255±13ab	245±13ab
	Fructose	207±15a	159±6ab	199±16a	161±29ab	87.48±19.40b	154±37ab	118±46ab	178±38ab	130±2ab	127±18ab	147±14ab
	Total	759	575	725	565	348	574	444	666	531	506	515
Fatty acids (μg/kg)	C12:0	105±3b	132±11ab	143±7a	150±15a	142±7a	142±2ab	140±12ab	140±14ab	135±0ab	139±9ab	136±3ab
	C14:0	144±7b	173±12ab	197±10a	166±13ab	171±5ab	171±1ab	160±1b	173±3ab	174±12ab	173±14ab	157±3b
	C14:1n5	122±6ab	145±14a	134±18ab	103±1b	101±0b	145±1a	105±8b	115±7ab	110±8ab	114±10ab	107±3b
	C16:0	627±3c	77.41±3.31abc	85.09±7.46ab	91.04±5.52a	79.00±3.09abc	74.01±1.15abc	72.08±2.96bc	76.25±4.25abc	76.76±3.11abc	73.51±8.01bc	67.96±1.28bc
	C16:1n7	423±12d	511±16abc	535±20a	504±11abc	436±4d	530±10ab	427±6d	500±14abc	457±12cd	455±12cd	475±28bcd
	C18:0	88.01±4.75a	103±7a	91.14±7.37a	107±17a	116±17a	81.87±0.77a	85.33±26.56a	101±2a	95.34±1.52a	96.70±18.89a	nd
	C18:1n9c	184±45b	166±21b	83.33±5.52b	754±112a	118±13b	98.67±19.94b	79.97±28.08b	107±24b	81.42±1.52b	108±26b	53.92±9.81b
	C18:2n6c	337±46b	296±2b	317±23b	609±61a	266±7b	300±8b	233±1b	316±34b	273±37b	248±22b	264±4b
	C18:3n3	67.18±0.01b	80.49±10.35ab	83.33±5.52a	nd	nd	nd	nd	nd	nd	nd	nd
	Total	2097	1684	1669	2484	1429	1543	1302	1528	1403	1407	1261
Free amino acids (μg/kg)	L-Asp	137±5bcd	153±11abc	148±0abc	128±14cd	172±12ab	179±7a	145±4abc	139±0abcd	128±12cd	159±4abc	98.52±21.25d
	L-Thr	81.99±5.83bcd	106±6ab	137±0a	80.75±5.39bcd	88.25±1.66bc	102±14b	51.08±14.19d	76.14±3.79bcd	67.44±15.25cd	80.18±5.22bcd	59.74±3.46cd

L-Ser	125±3bc	142±6b	169±0a	114±5c	117±4c	119±8bc	75.18±6.78de	88.30±1.04d	55.9±10.27ef	86.96±6.43d	41.04±3.29f
L-Glu	390±0d	486±10c	580±0b	265±0e	377±0d	263±12e	43.90±0.01f	68.95±0.02f	374±0.02d	273±0e	648±30a
L-Gly	25.8±0.39b	29.24±2.04a	26.76±0.01ab	16.40±0.10c	13.04±0.01e	13.51±0.01de	11.12±0.01e	15.86±.010cd	nd	nd	nd
L-Ala	50.01±0.01ab	43.54±1.15ab	47.53±0.01ab	37.30±0.61ab	33.49±8.40b	42.60±3.55ab	38.73±3.65ab	54.79±11.96a	33.55±4.39b	34.79±3.70ab	31.37±4.34b
L-Val	81.89±0.01bc	96.33±3.42b	126±0a	80.63±0.25c	77.83±5.07c	56.97±11.05d	1.13±0.36f	29.88±2.16e	5.43±0.05f	42.18±0.01de	4.17±0.12f
L-Cys	54.70±0.01a	5.32±0.01e	5.48±0.01e	7.05±0.47e	9.33±3.46e	36.59±1.02cd	44.28±3.03bc	30.47±4.32d	46.34±1.84ab	33.42±0.52d	50.35±4.34ab
L-Met	3.21±0.01c	1.64±0.01d	1.83±0.73d	2.09±0.01d	nd	nd	nd	3.84±0.01c	nd	6.54±0.01a	4.78±0.02b
L-Leu	29.13±0.01b	30.02±3.83ab	34.78±0.81a	19.03±1.24c	14.85±1.5cd	11.44±0.49de	10.66±0.06de	2.29±0.05f	6.32±0.01ef	17.94±0.03c	1.73±0.07f
L-Tyr	16.92±0.01c	20.88±0.03b	24.92±1.96a	14.02±0.02d	nd	nd	17.70±0.01c	20.40±0.03b	nd	20.91±0.01b	17.71±0.42c
L-Phe	25.52±0.01cd	29.73±4.62bcd	36.66±0.45abc	29.54±4.77bcd	37.73±5.87abc	40.25±1.33ab	28.59±2.83bcd	41.63±5.51ab	30.70±4.88bcd	46.60±0.01a	20.5±2.93d
L-Lys	19.67±0.45b	20.86±0.15a	17.43±0.43c	16.71±0.21c	13.90±0.01d	13.00±0.01e	9.57±0.03f	1.41±0.01g	nd	nd	nd
L-His	14.58±0.01c	24.38±1.52a	25.28±2.06a	18.51±1.34b	18.63±0.96b	2.52±0.24d	nd	nd	nd	nd	nd
L-Arg	89.97±5.76a	62.76±3.59b	65.54±1.46b	58.32±0.21b	55.77±1.62b	38.85±1.52c	34.95±1.03cd	39.06±4.20c	29.77±0.92cd	26.46±0.04d	26.69±2.63d
L-Pro	4422±112a	4489±236a	4637±692a	2771±136b	4506±156a	5260±284a	4846±104a	4619±71a	4866±60a	5420±13a	4746±421a
P-Ser	261±88cd	221±14d	203±0d	285±1bcd	333±0abcd	421±23.56ab	397±32abc	399±55abc	431±9a	451±1a	327±27abcd
Tau	20.30±3.15d	16.85±2.65d	19.96±0.01d	21.85±2.75cd	39.73±8.99bc	45.26±0.10ab	54.40±0.01ab	47.71±5.60ab	61.87±9.95a	53.75±0.10ab	62.57±3.26a
PEA	22.18±0.01d	13.86±0.55d	17.08±0.01d	12.51±2.61d	22.12±2.07d	52.46±0.87bc	65.86±0.01abc	48.03±3.32c	80.32±12.07a	52.3±8.53bc	70.82±3.78ab
L-Cit	nd	nd	18.36±0.01h	21.24±0.02g	26.8±0.02f	57.94±0.02e	65.44±0.01c	61.06±0.01d	91.40±0.01a	nd	75.85±0.01b

α-AAA	35.73±0.01a	36.50±0.01a	34.1±0.01a	19.59±2.99bc	20.08±0.01bc	30.93±0.03a	nd	35.60±0.01a	17.26±0.01bc	15.07±4.99c	23.92±0.01b
Cysthi	17.02±0.01b	17.2±1.67b	22.86±0.02a	15.98±0.01b	9.41±0.26cd	11.33±0.01c	8.11±0.01d	5.48±0.01e	1.49±0.25f	0.59±0.01f	0.12±0.01f
β-AiBA	40.73±0.88b	54.65±4.78a	56.00±0.46a	52.53±1.33a	47.84±1.35ab	38.91±1.42b	23.74±1.17cd	21.68±4.54cd	18.95±2.27de	28.54±2.49c	10.63±0.39e
γ-ABA	227±19a	258±10a	271±5a	241±18a	219±32a	220±15a	120±1bc	153±1b	90.90±0.78cd	137±0bc	62.77±3.46d
$EOHNH_2$	15.02±0.64b	17.56±0.03ab	19.36±2.01a	14.67±1.79b	17.09±2.27ab	nd	nd	nd	nd	nd	nd
Hypro	33.23±6.02ef	29.21±0.02f	24.66±0.31f	40.58±3.5de	44.89±0.07cd	58.98±1.99a	57.3±0.64ab	47.12±0.62cd	48.91±1.45bcd	50.50±1.29abc	45.13±0.38cd
Total	6240	6406	6771	4383	6315	7115	6150	6050	6485	7037	6430

Mean values with different lower-case letters in the same row correspond to significant differences at $p < 0.05$. Data are represented as the mean ± SD; "nd": Not detected.

C12:0: lauric acid, C14:0: myristic acid, C14:1n5: myristoleic acid, C16:0: palmitic acid, C16:1n7: palmitoleic acid, C18:0: stearic acid, C18:1n9c: oleic acid, C18:2n6c: linoleic acid and C18:3n3: α-linolenic acid.

3.3.2 Changes in contents of fatty acid during freeze drying

Fatty acids are the most important precursors for the formation of fruit aroma components. The linear aliphatic alcohols, aldehydes, ketones and esters are mainly derived from fatty acid oxidation (Schwab et al., 2008a). A total of 9 fatty acids were identified and quantified including lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1n5), palmitic acid (C16:0), palmitoleic acid (C16:1n7), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c) and α-linolenic acid (C18:3n3) (**Table 5-3**). These fatty acids were also found in other varieties of jujubes (J. Song et al., 2019). The fatty acid contents showed a trend of firstly increased from 2,097 μg/kg to 2,484 μg/kg (stage 0-3) and then decreased to 1,261 μg/kg (stage 10), with fluctuations during FD stages. Among these fatty acids, the contents of C12:0, C14:0, C16:0 and C16:1n7 increased slightly at the end of FD. And the others showed a decreased content after FD, especially C18:2n6c and C18:3n3, which were precursors of linear esters compounds through lipoxygenase pathway. This is also consistent with the result that the content of esters increased after FD.

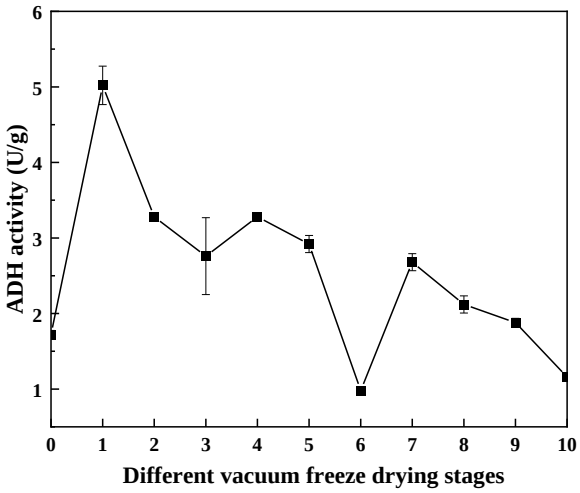
3.3.3 Changes in contents of free amino acids (FAAs) during freeze drying

From **Table 5-3**, a total of 26 free amino acids were detected in all red jujube samples. The total free amino acids showed an increasing tendency firstly, and then decreased to 4,383 μg/kg at stage 3, finally increased and kept a range from 6,050 μg/kg to 7,115 μg/kg during stage 4-10. FAAs had different changes in red jujube during the whole FD stages. After FD, glycine (Gly), valine (Val), histidine (His), lysine (Lys), Leucine (Leu), cystathionine (Cysthi) and ethanolamine (EOHNH2) were lost more, with a loss ratio of more than 90%, followed by serine (Ser), arginine (Arg), *β*-aminoisobutyric acid (*β*-AiBA) and *γ*-aminobutyric acid (*γ*-ABA), with a loss of 65%~75%, aspartic acid (Asp), threonine (Thr), alanine (Ala), phenylalanine (Phe), *α*-aminoadipic acid (*α*-AAA) and cysteine (Cys) were lost less than 40%. In addition, proline (Pro), tyrosine (Tyr), serine (Ser) and hydroxyproline (Hypro) increased 4%~40%, while, methionine (Met), glutamic acid (Glu), citrulline (Cit), *o*-phosphoethanolamine (PEA) and taurine (Tau) increased over 50%. Combined the original content of raw red jujube, data fluctuation and ratio of FAAs, Ser, Gly, Pro, Val, Cys, Arg, Glu, Lys and Leu could be potential precursors for the characteristic aroma of freeze-dried red jujube. These amino acids might involve in Maillard reaction, Strecker degradation, decarboxylation or deamination and other

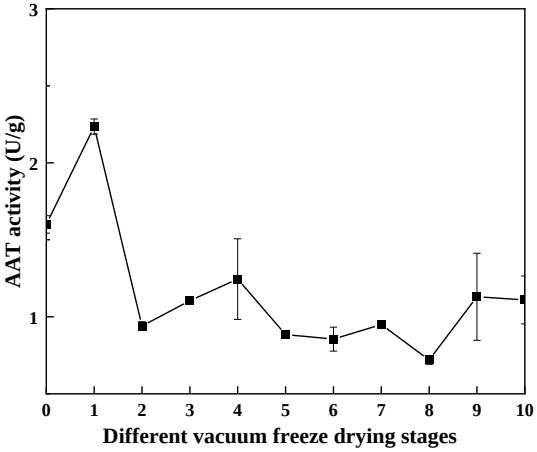
reactions in the thermal reaction process, and form various volatile compounds (El Hadi et al., 2013; Schwab et al., 2008a). Pyrazine compounds with roasty and nut notes obtained by Strecker degradation of Cys or thermal reaction of Lys, Gly, Ser, Val, Leu and Arg (Adams & De Kimpe, 2007; Deng et al., 2022; Wang et al., 2021).

3.4 Changes in key enzyme activities in red jujube during freeze drying

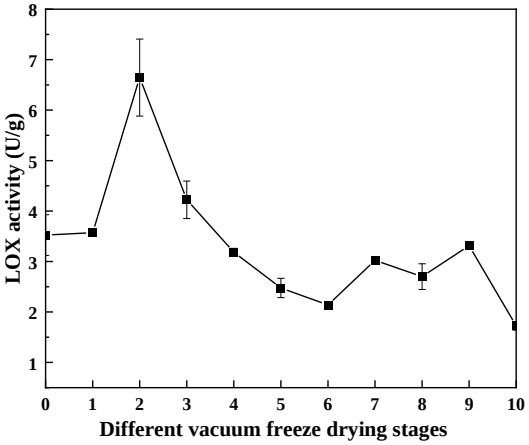
Linear-chain aliphatic alcohols, aldehydes, ketones and esters, are commonly derived from the oxidative degradation of fatty acids and are generally formed by LOX pathways in fruits (Wu et al., 2020). LOX, ADH, and AAT are important for the LOX pathway, which results in the synthesis of volatile compounds in red jujube samples. As displayed in **Figure 5-3 (a-c)**, the activities of ADH and AAT were reached the highest values with 5.02 U/g and 2.24 U/g at the stage 1, and the highest value of LOX was 6.65 U/g at the stage 2. The activity changes of LOX, ADH and AAT showed a consistent trend, increased initially and then declined. Though the enzyme activities had some fluctuation, the activities of ADH, AAT and LOX were lost 76.9%, 50.3% and 74.0% at the stage 10, respectively. In general, the enzyme activity is higher between 30-45 ℃ (Liu et al., 2013), while the temperature was higher than 57 ℃ after stage 2, which could cause the enzyme activity to decrease or even inactivate (**Figure 4-1)**.



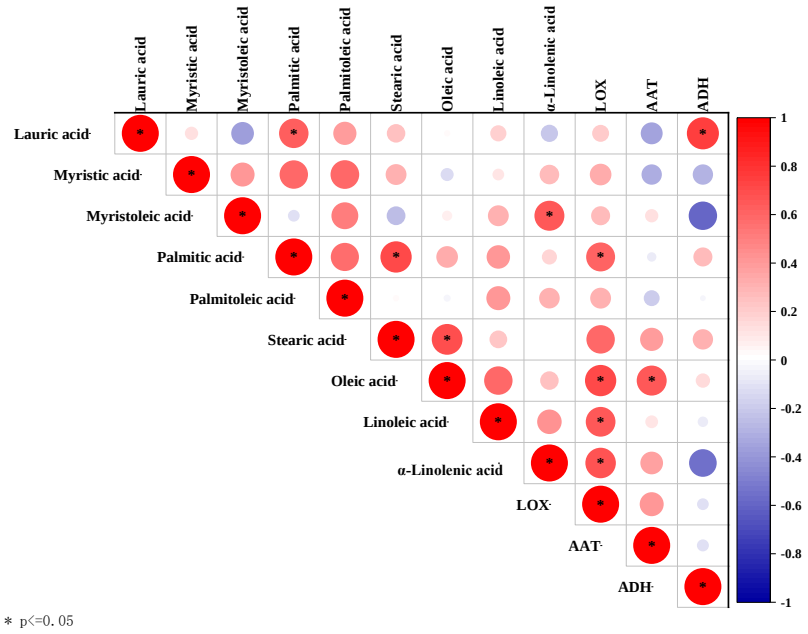
(a)


AAT activity (U/g)
Different vacuum freeze drying stages

(b)


LOX activity (U/g)
Different vacuum freeze drying stages

(c)



(d)

Figure 5-3 The enzyme activity changes of lipoxygenase (LOX) (a), alcohol dehydrogenase (ADH) (b) and alcohol acyltransferase (AAT) (c) during the different stages of freeze drying, and the correlation between enzyme activities and fatty acids (d).

From **Figure 5-3(d)**, LOX activity showed a significantly positive correlation with palmitic acid, oleic acid, linoleic acid and α-linolenic acid; ADH showed a positive correlation with lauric acid; AAT showed a positive correlation with oleic acid. In general, LOX recognizes the 1,4-pentadiene structure of linoleic acid and linolenic acid in unsaturated fatty acids to make them undergo oxidation and form hydroperoxide fatty acids, and hydroperoxide forms hexanal or hexenal under the action of hydroperoxide lyase (HPL) (Schwab et al., 2008a). Under the action of ADH, the corresponding alcohol is formed, such as, (*E*)-2-hexen-1-ol and the alcohol forms the corresponding ester, such as (*E*)-ethyl hex-2-enoate under the action of AAT (Guo et al., 2022).

3.5 Correlation between aroma compounds and precursors and enzyme activities in red jujube during freeze drying

3.5.1 Correlation analysis between classes of aroma compounds and precursors, enzyme activities and temperature

In order to explore the correlation between classes content of aroma compounds, the Spearman correlation analysis was established (**Figure 5-4** upper right). A correlation was also established between the class content of aroma compounds and precursors and temperature using the Mantel test (**Figure 5-4**, bottom left). Spearman correlation showed that esters content was negatively correlated with the content of acids, lactones, pyrazines, aldehydes and ketones. The content of pyrazines, ketones, acids and aldehydes were positively correlated. Butane-2,3-dione and 3-hydroxybutan-2-one with creamy and sweet notes in red jujube as α-dicarbonyl compounds, could participate in the Maillard reaction and form pyrazines with roasty and nut notes (Xiao et al., 2018). This result elucidated the positive correlation between ketones and pyrazines, also explained the aroma profile transformed from creamy and sweet to roasty after FD. With the Maillard reaction occurred, pyrazines as the products of Maillard reaction, ketones and acetic acid as the intermediate products would be produced (Gong et al., 2021). That could be explained the positive correlation between pyrazines, ketones, acids and aldehydes.

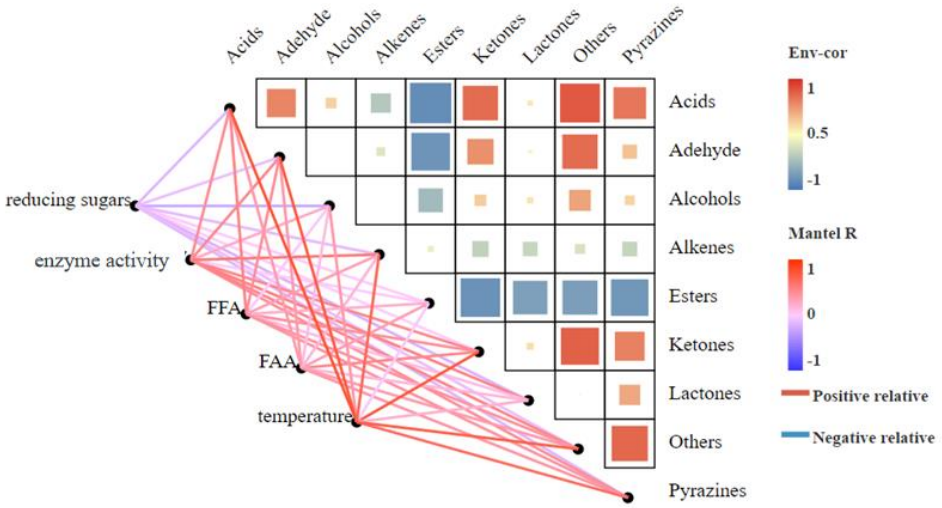


Figure 5-4 Correlation analysis between classes of volatile compounds and precursors, enzyme activities and temperature by Mantel test. The upper right diagram showing the Spearman correlation of different classes of aroma compounds. A color gradient denotes the Spearman's correlation coefficients. The bottom left graph shows the Mantel test between effect parameters (reducing sugar, enzyme activies, FFA, FAA, and temperature) and different classes of aroma compounds mentioned above. FFA, free fatty acids; FAA, free amino acids.

Results of the Mantel test indicated that enzyme activity, fatty acids and free amino acids had significant correlations ($p \leq 0.05$) with aldehydes, ketones, acids, lactones, pyrazines, alkenes and others. Amino acids and fatty acids could not only affect the volatile compounds alone, but also their interactions could affect the overall aroma to a great extent, which is mainly played by Maillard reaction. In Maillard reaction, amino compounds could be provided by amino acids, while carbonyl compounds could be converted from reducing sugar or fatty acids (Hou et al., 2017). In addition, some volatile oxidation products of fatty acids, such as acids, ketones, alcohols, would also react with the intermediate products of Maillard reaction to generate flavour compounds and contribute to the overall aroma. They might generate some heterocyclic compounds with long alkyl substituents, such as pyridines, pyrazines and so on (Liu et al., 2020). In addition, temperature had a highly significant correlation ($p \leq 0.01$) with the aldehydes, ketones, acids, lactones, pyrazines, alkenes and others. That illustrated the temperature was also a key influencing factor in the aroma formation during FD which is a complex process involved enzyme reaction and non-enzyme reaction. Li et al. (2022) also found that temperature was an important parameter in aroma formation during drying processing of shiitake mushrooms. And based on PCA analysis, they inferred the aroma formation was dominated by enzymatic reactions in the pre-drying period and by non-enzymatic reactions in the post-drying period, which were all driven by temperature. In our study, combined the correlation value, the influence degree of factors on the aroma formation of freeze-dried jujube was ranked as temperature > enzyme activity > fatty acids > amino acids (**Figure 5-4**).

3.5.2 Correlation analysis between aroma-active compounds and flavour precursors, enzyme activities

Based on the above analysis results, the correlation network among aroma-active compounds (OAV>1), flavour precursors (including 3 fatty acids, 9 free amino acids and 3 sugars), and enzyme activities was constructed using Cytoscape (v.3.8.2) based on the Spearman correlation analysis. Spearman correlation coefficients and p values were calculated and shown in **Figure 5-5**. There were 212 significant ($p < 0.05$) and strong ($|r| > 0.6$) correlations between the aroma-active compounds and flavour precursors and enzyme activities. The letter A~I stand for alcohols, aldehydes, ketones, acids, esters, lactones, pyrazines, alkenes, and others, respectively.

From **Figure 5-5**, there were 3 fatty acids related to volatile compounds, of which the number of aroma-active compounds related to α-linolenic acid was the most (18), followed by linoleic acid (9) and oleic acid (6). That might be due to these fatty acids have unsaturated double bond, which could more easily form aroma compounds by oxidizing reaction. In addition, there were 9 free amino acids related to volatile compounds. The number of aroma-active compounds related to Lys (23) and Leu (23) was the largest, followed by Arg (21), Ser (20), Val (20) and Gly (19), Pro (15), Glu (8) and Cys (3). This result was consisted with section 3.2.3, these amino acids could be identified as the main FAAs precursors for the aroma-active aroma compounds of freeze-dried red jujube. Glucose had more correlations with aroma-active compounds among the 3 sugars, and LOX had greater correlation with aroma-active compounds than ADH and AAT.

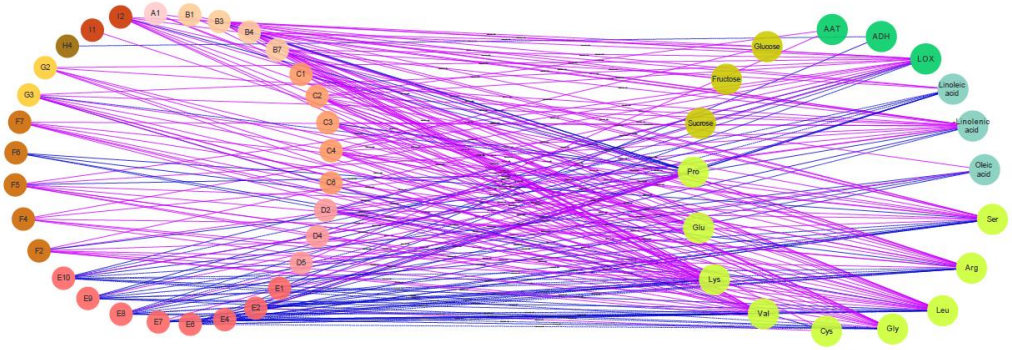


Figure 5-5 Spearman correlation networks showing relationships between aroma-active compounds (OAV>1) and flavor precursors, enzyme activities in red jujube during freeze drying stages. The left-hand circle represents the aroma-active compounds, and the right-hand circle represents the main flavor precursors and enzyme activities in the red jujube during freeze drying. The purple and blue lines respectively represent the positive and negative correlation between the aroma compounds and flavor precursors, enzyme activities. And correlation coefficients between them were calculated using values from all samples. Only significant correlations ($|r| > 0.6$, $p < 0.05$) are indicated, and line thickness represents the correlation coefficients of interactions. (For interpretation of the references to color and letter in this figure, the reader is referred to the web version of this article.)

As compounds that contribute greatly to the aroma of freeze-dried jujube, pyrazine compounds were mainly negatively correlated with Pro and Cys, and positively correlated with Gly, Lys, Val, Leu, Arg and Glu. This is consistent with Yu et al., (2021) and Kocadağlı et al., (2021). In addition, there was also a significant positive

correlation with α-linolenic acid and LOX, which might be due to the substances produced by fatty acids in the oxidation process involved in the Maillard reaction. Similar to pyrazines, precursors associated with ketones included 8 amino acids, 2 fatty acids and 1 reducing sugar, LOX and AAT also showed correlation with ketones. Among them, Arg, Lys, Leu, Val, Pro, Glu, Ser, Gly and α-linolenic acid were correlated with 3-octanone, 3-hydroxybutan-2-one, and oct-1-en-3-one. Ketone compounds showed a declined trend during the FD process, indicating that ketones were reactant during the FD process, and might be converted to other volatile compounds such as pyrazines or carboxylic acids (Xiao et al., 2018).

There were 9 amino acids, 3 fatty acids and LOX significantly correlated with esters. Among these aroma-active compounds, ethyl decanoate, methyl dodecanoate and methyl decanoate were identified as the aroma-active compounds of "Huizao" and freeze-dried "Huizao". In addition, ethyl dodecanoate and ethyl heptanoate were identified as aroma-active compounds in freeze-dried "Huizao", ethy octanoate was also produced after FD. Generally, esters could be divided into two categories, one is acetyl coenzyme A and higher alcohols to produce acetates, the other is fatty acids and ethanol to produce fatty acid ethyl esters. Higher alcohols are mainly derived from amino acid catabolism, while acetyl coenzyme A could be produced through various pathways, including amino acid metabolism and fatty acid oxidation, and LOX is an important pathway for fatty acid oxidation (Schwab et al., 2008a). Therefore, it could well explain the strong correlation between the dynamic changes of esters and amino acids, fatty acids and LOX enzyme activity.

Similar to esters, lactones were mainly related to α-linolenic acid, LOX enzyme activity and 8 amino acids. Chemically, they are cyclic esters formed by intramolecular condensation of hydroxy fatty acids (El Hadi et al., 2013). The typical lactones in "Huizao" and freeze-dried "Huizao" were γ-lactones. The 5-propyloxolan-2-one was identified as the key aroma-active compounds in "Huizao" before and after freeze-drying, and the 5-heptyloxolan-2-one was identified as the key aroma-active compounds of freeze-dried "Huizao". In fact, most of the hypotheses on the biosynthesis of fruit lactones involved two main pathways for fatty acids, β-oxidation and LOX to produce aroma compounds. Although the importance of these compounds in fruit aroma, there is a lack of enzymatic research in fruit (El Hadi et al., 2013).

There were also many precursors related to aldehydes, including 2 fatty acids and

8 amino acids, and 3 sugars. The number of precursors related to (*E*)-2-heptenal and (*E*)-2-octenal were the most (11), followed by decanal (10), and most of the precursors were amino acids. Furthermore, these three aldehydes were not detected after FD stage 4, indicating that aldehydes were intermediate products during the FD process. There were few precursors related to alcohols, including Cys, Leu and *α*-Linolenic. The content of alcohols increased slightly after FD, oct-1-en-3-ol might be derived from fatty acids under the action of ADH, and 2,3-butanediol might be originated from metabolism of pyruvate (Zhang et al., 2020).

4. Conclusion

A total of 30 aroma-active compounds of 53 aroma compounds were detected in all red jujube samples during FD processing, and the aroma content increased 32.7% after FD. From stage 3 in FD processing, the aroma profile of freeze-dried red jujube was transformed from sweet dominated to roasty dominated. In addition, there were 9 FAAs (Ser, Gly, Pro, Val, Cys, Arg, Glu, Lys and Leu) and 3 FFAs (oleic acid, linoleic acid and *α*-linolenic acid) selected as key aroma precursors; and LOX play an important role in aroma formation of freeze-dried red jujube. Through analysis of precursors and enzyme activities combined correlation analysis, glucose and FAAs involved in non-enzymatic reactions, they had the main correlation with the formation of esters, pyrazines and furfural; and the FFAs and LOX involved in lipid oxidation reactions, they had the main correlation with the formation of alcohols, aldehydes and lactones. In addition, the influence degree of factors on the aroma formation of freeze-dried jujube was ranked as temperature > enzyme activity > fatty acids > amino acids. The multi-stage and variable-temperature procedure of FD enhanced lipid pyrolysis reaction and non-enzymatic reaction efficiency, which significantly improved the aroma of red jujube.

Furthermore, this study provides a better understanding of how the aroma of red jujube is modified during the freeze-drying process and the origin of these olfactory changes. And reveals which flavour precursors are most important for the development of the characteristic red jujube flavour sought by consumers, thereby enabling the selection of the most suitable red jujube variety for the targeted technological transformation.

6

Chapter VI. Modelling alkylpyrazine formation in red jujube matrix under controlled conditions of pH and temperature

In our previous chapters, alkylpyrazines played a significant role in aroma of red jujube and its processed products, contributed the nutty or roasty notes. In most of published literature, the studies on formation pathway for pyrazine were generally based on an alkaline liquid-state model system and higher temperature (more than 100 °C), whereas red jujube are a solid-state with a pH of 5.5 and the actual processed temperature is lower than 100 °C. Therefore, the aim of this chapter was to establish a solid-state model system at pH 5.5 and compare to another one with pH 7.8, and the reaction condition was applied, which was similar with pilot scale freeze-dried one, to investigate the pathway for the formation of alkylpyrazines in freeze-dried red jujube.

This article has not been published yet.

Abstract: The formation of alkylpyrazines is influenced by substrates, temperature, pH and other factors. To investigate alkylpyrazine formation based on red jujube matrix and under the condition of freeze drying, solid-state models of varying pH were established, and volatile compounds were detected during the reaction process. The correlation between these compounds was also analysed. The content of pyrazines at pH 7.8 was only 11.34% higher than that in the pH 5.5 model. In the pH 5.5 model, butane-2,3-dione, pentane-2,3-dione, pyruvic acid, isopropyl alcohol, acetone and 2-hydroxypropionic acid had a significant correlation with 2-ethyl-3,5-dimethylpyrazines (3,5-EDMP). In red jujube matrix, the dominant formation of 3,5-EDMP is condensation of 2-aminopentan-3-one produced by pentane-2,3-dione and aminoacetone produced by the Strecker degradation of methylglyoxal to form dihydropyrazine, which is then oxidised to form 3,5-EDMP. A verification experiment in a real model system illustrated that the aroma of processed red jujube products can be modulated by adding relevant aroma precursors.

Keywords: Maillard reaction, solid-state model, pH, red jujube matrix, pyrazines formation

1. Introduction

Jujube (*Ziziphus jujuba* Mill.) belongs to the family Rhamnaceae, and red jujube can be used not only as a fruit but also in Chinese folk medicine. Red jujube contains rich nutritional and functional ingredients, such as triterpenoid acids, flavonoids and phenolic acids (Song et al., 2019). Aroma is another reason for the popularity of red jujube, which contains esters with fruity and sweet notes, acids with sour notes, ketones with creamy notes, aldehydes with green notes and pyrazines with nutty notes. These compounds confer a unique aroma on red jujube (Gou et al., 2022). Also, red jujube is rich in aroma precursors, such as amino acids, fatty acids and reducing sugars (Gou, et al., 2023a), which can provide important substrate sources for the aroma of processed red jujube products. In addition to being eaten fresh, most red jujube will be processed via methods such as steaming, hot air drying, heat pump drying, freeze drying, baking, frying and so on. Especially, freeze-dried red jujube has become a popular product, with an improved appearance, colour and aroma. In a practical application, a multi-stage and variable-temperature procedure was applied in industry using a high-temperature heating plate (85~65 °C) in the process of freeze drying red jujube (Gou, et al., 2023b). It is possible that the Maillard reaction occurred during this process, and pyrazines were identified as the key aroma compounds (Gou, et al., 2023b).

Pyrazines are a class of six-membered heterocyclic compounds containing two nitrogen atoms at the 1 and 4 positions (Keyhani & Yaylayan, 1996). They have the characteristics of low vapor pressure, high volatility and a low threshold value and are widely found in various natural foods, heat-processed foods and fermented foods such as, coffee, cocoa and cake (Al Tamimi et al., 2023; Arsa & Puechkamutr, 2022; Library et al., 2012). Pyrazines can be classified into many different compounds depending on the substituents carried by the four carbon atoms on the aromatic ring. In a previous study, the pyrazines detected in red jujube and its product were mainly alkylpyrazines, including 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, tetramethylpyrazine and 2-ethyl-3,5-dimethyl-pyrazine. These compounds contributed the nutty or roasty notes to red jujube and its products and play an important role in their overall aroma profiles (Gou et al., 2022; Gou, et al., 2023b).

Studies have shown that pyrazines are mainly generated from Maillard reactions during food production processing, and α-carbonyl compounds are important

precursor compounds of pyrazines, which are degradation products of reducing sugars (Shu, 1998). The α-carbonyl compounds can react with amino acids to form α-aminocarbonyl compounds and Strecker aldehydes. Furthermore, dihydropyrazine formed by two α-aminocarbonyl compounds condensed and were converted to the corresponding alkylpyrazine via oxidative pathways. Alternatively, deprotonated dihydropyrazine can react with carbonyl compounds to form the corresponding alkylpyrazine (Deng et al., 2022; Guerra & Yaylayan, 2012; Ma et al., 2022; Mei et al., 2007; Scalone et al., 2015). The formation of pyrazines is involved the Maillard reaction, which is influenced by many factors, such as the types of substrate, water activity, temperature, time and pH (Lotfy et al., 2021; Shen et al., 2019; Xia et al., 2022; H. Yu et al., 2021). Much of the literature on the factors affecting the formation of pyrazines is based on a liquid model system and reaction temperatures above 100 ^{o}C (Deng et al., 2022; Guerra & Yaylayan, 2012; Guo et al., 2018; Ma et al., 2022; Mei et al., 2007; Scalone et al., 2015; Van Lancker et al., 2012; Wang et al., 2021).

However, the temperature is not over 100 ^{o}C during the freeze drying of red jujube, and the red jujube is in the solid state. Besides, the initial pH of the Maillard reaction greatly influences production, and the pH of red jujube (5.5) is lower than that favourable for pyrazine formation. Therefore, this study intends to establish a solid-state model system at pH 5.5 and compare it to another one at pH 7.8. The highest commercial plate temperature (85 °C) and treatment time (10 h) were used for the reaction conditions to investigate the pathway for the formation of alkylpyrazines in freeze-dried red jujube.

2. Materials and methods

2.1. Materials and chemicals

All red jujube (*Zizyphus jujuba* cv. Huizao) were purchased from Akesu, the Xinjiang Uyghur Autonomous Region, China. Red jujubes without mechanical damage were stored immediately in the laboratory at 4 ^{o}C until use. The water content of "Huizao" was 25.57%, and the pH was 5.5.

D-Glucose (99%), *L*-glycine (99%), *L*-arginine (99%), *L*-lysine (99%), *L*-serine (99%), *L*-proline (99%), butane-2,3-diol (≥ 98%), furfural (≥ 98%), butane-2,3-dione (≥ 98%), pentane-2,3-dione (≥ 98%), 3-hydroxybutan-2-one (≥ 98%), 6-methylhept-5-en-2-one (≥ 98%), acetic acid (≥98%), 2-ethyl-5-methylpyrazine

(≥98%), 2-ethyl-6-methylpyrazine (≥98%), trimethylpyrazine (≥ 98%), 2,6-diethylpyrazine (≥ 98%), 2-methylpyrazine (≥ 98%), 2-ethyl-3,5-dimethylpyrazine (3,5-EDMP) (≥ 98%), styrene (≥ 98%), 2-cyclohexene-1-one (≥98%) and *n*-alkane (C5-C40) were purchased from Shanghai Yuanye (Shanghai Yuanye Bio-Technology Co., Ltd, Shanghai, China) and Macklin (Shanghai Macklin Biochemical Co., Ltd, Shanghai, China).

2.2. Preparation of odourless matrix

Firstly, the kernel of red jujube was removed, and the remaining part was cut into 5 mm slices. Then 400 g of red jujube slices were mixed with 4 L of deionized water for ultrasonic treatment at 40 kHz for 1 h. The obtained extract was drained, and the above steps were repeated a total of 6 times, to obtain red jujube slices without sugar or organic acid (Hou et al., 2021). The aroma compounds of red jujube slices were removed with a mixture of ether and pentane (v:v = 1:1). Subsequently, the samples were dried at 20 °C for 48 h using a freeze dryer (Alpha 1-4 LD plus, Marin Christ, Osterode, Germany) until nothing was detected by GC-MS.

2.3. Establishment of solid-state model system

A Maillard model system was constructed with glucose, different amino acids and odourless matrix. Specifically, glucose (5 g) + *L*-glycine (5 g), glucose (5 g) + *L*-arginine (5 g), glucose (5 g) + *L*-lysine (5 g), glucose (5 g) + *L*-serine (5 g) and glucose (5 g) + *L*-proline (5 g) were dissolved in 35 mL phosphate buffer solution at pH 7.8 and pH 5.5, respectively. Then, 8 g of odourless matrix of red jujube was added to these eight mixture solutions and absorbed all the solution. Finally, the model reaction was heated in an oven at 85 °C for 10 h. Besides, the glucose + lysine systems at pH 5.5 (model 1) and pH 7.8 (model 2) were heated for 2, 4, 6, 8 and 10 hours.

2.4. Verification experiment of real system

The model 3 consisted of glucose (5 g), *L*-lysine (5 g), 35 mL phosphate buffer solution of pH 5.5 and 8 g of odourless matrix of red jujube reacted under industrial freeze-drying conditions. In addition, the original red jujube slices without added glucose and amino acid served as the control sample, and the 30 g of original red jujube slices with 5 g *L*-lysine added to 20 mL water served as model 4; these groups were also reacted under the same conditions. The specific conditions of freeze

drying are described in Gou et al. (2023).

2.5. HS-SPME/GC–MS analysis of aroma composition

The DVB/CAR/PDMS fibre (65 μm) was applied to extract the volatile compounds according to the method of Gou et al. (2022). The volatile compounds in the sample were identified by GC-MS using an 8890 GC System (5977B MSD) equipped with a DB-Wax column (60 m × 0.25 mm, 0.25 μm). The oven parameters were as follows: the initial temperature was held at 40 ℃ for 3 min, heated to 120 ℃ at 4 ℃/min, then rose to 200 ℃ at 10 ℃/min, and held for 5 min. The helium (purity = 99.99%) was used as the carrier gas at 1.0 mL/min. The ionization method was Electron-impact (EI), and the fragments created by EI were scanned from 35 to 550 m/z.

2.6. Statistical Analysis

The software SPSS version 20.0 (Armonk, NY: IBM Corp.) was performed for statistical analysis. Significant differences were presented by Duncan's test ($p < 0.05$). Results were performed by mean ± standard deviation. The data were illustrated using Origin 2022 (OriginLab Corporation, Northampton, MA). A clustering correlation heatmap was performed using the OmicStudio tools at https://www.omicstudio.cn.

3. Results and discussion

3.1. Amino acids selection for pyrazines production

2-Ethyl-3,5-dimethylpyrazine (3,5-EDMP), 2,6-dimethylpyrazine, 2-ethyl-6 methylpyrazine, 2,6-diethylpyrazine and tetramethylpyrazine are the important aroma compounds in freeze-dried red jujube, which are produced by a Maillard reaction under the multi-stage and variable-temperature freeze-drying procedure (Gou, et al., 2023a). In our previous study, *L*-Ser, *L*-Gly, *L*-Pro, *L*-Arg and *L*-Lys were the main free amino acids and could react with glucose to generate pyrazines in red jujube (Gou, et al., 2023b). To explore which amino acids could synthesize more pyrazines, amino acid selection experiments were conducted by establishment of a solid-state model system. As shown in **Table 6-1**, Pro and Ser yielded the lowest content (18.15 and 42.69 μg/kg) and number of species (1 and 4) of pyrazines, whereas lysine and arginine, as basic amino acids, are more likely to undergo the

Maillard reaction to produce pyrazines (Library et al., 2012). The most species of pyrazines were generated in the Arg-glucose model, but the total pyrazine content (369 μg/kg) was lower than in the Lys-glucose model (3,050 μg/kg). Besides, 3,5-EDMP was the key aroma-active compound in freeze-dried red jujube, which also had the highest content in the Lys-glucose model (361 μg/kg). Therefore, Lys was selected, which is conducive to subsequent experimental detection.

Table 6-1 Yields of pyrazines in different amino acid and glucose models.

Contents（μg/kg）	Arg	Gly	Lys	Ser	Pro
2-Methylpyrazine	16.85±1.68b	nd	46.32±4.6a	6.86±0.48c	nd
2,5-Dimethylpyrazine	190±16b	82.38±7.09c	2165±172a	nd	nd
2,6-Dimethylpyrazine	24.00±2.31b	11.38±1.14c	72.15±5.53a	9.02±0.2c	18.15±1.41bc
2-Ethylpyrazine	11.69±1.63b	nd	nd	18.87±0.99a	nd
2-Ethyl-6-methylpyrazine	79.71±5.02b	nd	218±18a	7.93±0.27c	nd
2,3,5-Trimethylpyrazine	27.37±2.65c	93.13±8.91b	152±14a	nd	nd
3-Ethyl-2,5-dimethylpyrazine	nd	nd	19.34±1.31	nd	nd
2-Ethyl-3,5-dimethylpyrazine	4.37±0.41c	18.6±0.37b	361±21a	nd	nd
2,6-Diethylpyrazine	nd	18.86±0.53	nd	nd	nd
Tetramethylpyrazine	nd	19.96±1.69	nd	nd	nd
2-Ethenyl-6-methylpyrazine	8.99±0.32	nd	nd	nd	nd
2-Methyl-6-[(*E*)-prop-1-enyl]pyrazine	5.99±0.4b	nd	17.43±1.8a	nd	nd
Total	**369**	**244**	**3050**	**42.69**	**18.15**

3.2 GC-MS analysis during reaction processing at different pH

To illustrate the formation of pyrazines during freeze drying based on the red jujube matrix, a solid-state model of pH 5.5 (model 1) was established and volatile compounds formed during the reaction were detected. Beyond that, a solid-state model of pH 7.8 (model 2) was also established for comparison. A total of 51 (pH 5.5) and 44 (pH 7.8) volatile compounds were detected by GC-MS in different pH models. These compounds were alcohols, aldehydes, ketones, acids, esters, furans, furanones, pyrazines and other heterocyclic compounds. The content of total volatile compounds at pH 5.5 (17,790 μg/kg) was higher than that at pH 7.8 (12,411 μg/kg) at the end of the reaction, while the content of pyrazine was lower at pH 5.5 than at pH 7.8 (3,712 μg/kg at pH 5.5 and 4,133 μg/kg at pH 7.8) (**Table 6-2 and Table 6-3**). However, the content of pyrazine in the pH 7.8 model was only 11.34% higher than that in the pH 5.5 model. This may be due to the fact that lysine is an alkaline amino acid, so the effect of pH on pyrazine formation is not very significant (Library

et al., 2012). Both in pH 5.5 and pH 7.8, 2,5-dimethylpyrazine was the most abundant pyrazine, suggesting that Strecker degradation produced higher amounts of aminopropanone (Guo et al., 2018). In addition, furans and furanones showed a higher content of pH 5.5 (160 μg/kg) compared to pH 7.8 (129 μg/kg). These results revealed that low pH favoured the formation of furans and furanones, whereas pyrazines showed an increase at high pH, which was consistent with reported references (Lotfy et al., 2021; A. N. Yu & Zhang, 2010).

Table 6-2 The content of volatile compounds during reaction in pH 5.5 mode.

Numbers	Compounds	LRI(a/b)	Contents（μg/kg）				
			2 h	4 h	6 h	8 h	10 h
	Alcohol						
A1	Isopropyl alcohol	912/935	35.07±3.32a	5.94±0.26b	5.79±0.91b	6.42±0.6b	n.d
A2	Ethanol	925/933	126±11c	338±19b	121±1c	404±10a	n.d
A3	2-Methylpropan-1-ol	1119/1094	64.03±0.22a	36.69±2.72b	16.79±1.12c	n.d	n.d
A4	2-Ethylhexan-1-ol	1483/1517	67.24±4.3a	55.69±5.09a	16.75±0.99b	17.98±0.18b	27.18±2.28b
A5	Butane-2,3-diol	1554/1553	20.88±0.64a	n.d	7.13±0.67b	n.d	n.d
A6	Propylene glycol	1597/1603	35.26±3.06b	721±15a	9.11±0.69b	n.d	5.11±0.05c
A7	2-Furanmethanol	1667/1635	n.d	n.d	n.d	n.d	5.78±0.45
	Total		**348**	**1157**	**177**	**428**	**38.07**
	Aldehyde						
B1	Methylglyoxal	970/-	38.00±0.52a	30.83±1.76b	14.1±0.87c	n.d	n.d
B2	2-Methylpentanal	1104/-	21.83±1.59c	181±7a	5.08±1.04c	143±9b	147±4.b
	Total		**59.83**	**212**	**19.18**	**143**	**147**
	Ketone						
C1	Acetone	834/813	1315±92a	127±9b	125±8b	133±13b	n.d
C2	4-Hydroxybutan-2-one	873/-	15.91±1.55a	n.d	10.97±0.3b	n.d	n.d
C3	Butane-2,3-dione	964/979	464±38	n.d	n.d	n.d	n.d
C4	2-Methylpentan-3-one	1005/1000	29.34±2.31	n.d	n.d	n.d	n.d
C5	4-Methylpentan-2-one	1006/1008	1441±112a	n.d	n.d	n.d	193±3b
C6	Pentane-2,3-dione	1055/1062	20.37±1.71	n.d	n.d	n.d	n.d
C7	2-Hexanone	1100/1124	n.d	829±70b	410±29c	879±12b	1893±18a
C8	2-Heptanone	1174/1180	n.d	246±62a	114±17ab	202±15a	245±37a
C9	Hydroxyacetone	1275/1290	85.72±6.26a	29.74±1.07b	16.38±0.43c	15.17±0.16c	7.65±0.35c
C10	3-Hydroxybutan-2-one	1283/1286	521±33a	380±11b	135±2d	215±3c	121±10d
C11	2-Octanone	1294/1309	9481±670ab	12747±1250a	6192±128b	10350±580ab	10455±578ab
C12	6-Methyl-5-hepten-2-one	1365/1342	n.d	56.27±4.94a	n.d	n.d	22.6±1.99b

C13	2-Nonanone	1402/1386	53.86±3.51a	46.56±4.58ab	24.58±2.08b	46.11±3.81ab	56.55±3.91a
C14	2-Decanone	1482/1493	262±25a	151±14b	150±9b	173±8ab	175±5ab
C15	2-Undecanone	1595/1599	n.d	n.d	n.d	n.d	11.93±0.65
	Total		**13689**	**14613**	**7178**	**12013**	**13181**
	Acids						
D1	Pyruvic Acid	1230/-	48.05±2.06a	9.72±0.55b	12.88±0.83b	n.d	n.d
D2	Acetic acid	1460/1429	133±9b	81.55±8b	533±26a	253±17b	272±2b
D3	2-Methylpentanoic acid	1764/1746	n.d	n.d	n.d	n.d	4.16±0.02
D4	2-Hydroxypropionic acid	2159/-	48.08±0.69	n.d	n.d	n.d	n.d
	Total		**229**	**91.27**	**546**	**253**	**276**
	Esters						
E1	Methyl pyruvate	1217/-	n.d	n.d	59.82±3.93b	68.24±1.58a	n.d
	Total		**n.d**	**n.d**	**59.82**	**68.24**	**n.d**
	Pyrazines						
F1	2-Methylpyrazine	1265/1274	117±2a	55.9±1.43bc	32.27±2.54d	46.18±3.65cd	61.86±2.11b
F2	2,5-Dimethylpyrazine	1321/1332	3591±256a	1195±106c	2218±173b	3047±26a	2912±197ab
F3	2,6-Dimethylpyrazine	1348/1440	72.01±5.53b	158±15a	54.81±0.78b	63.67±5.9b	64.24±1.8b
F4	2-Ethyl-5-methylpyrazine	1378/1399	n.d	91.08±8.15b	85.37±1.5b	110±3ab	123±9a
F5	Trimethylpyrazine	1403/1413	82.36±6.35ab	82.82±7.41ab	75.58±5.16b	101±6a	97.93±2.23ab
F6	3-Ethyl-2,5-dimethylpyrazine	1430/1455	n.d	n.d	n.d	110±1b	167±6a
F7	2-Ethyl-3,5-dimethylpyrazine	1464/1464	n.d	145±13ab	130±2b	198±9a	180±15ab
F8	2-Ethenyl-6-methylpyrazine	1489/1485	n.d	n.d	n.d	n.d	31.04±1.41
F9	2-Ethenyl-5-methylpyrazine	1516/1493	n.d	n.d	n.d	n.d	19.49±1.3
F10	3,5-Dimethyl-2-(3-methylbutyl)pyrazine	1530/-	n.d	n.d	n.d	n.d	55.92±2.81
F11	2-Acetyl-3-methylpyrazine	1629/1640	n.d	n.d	6.41±0.35	n.d	n.d
	Total		**3862**	**1728**	**2602**	**3676**	**3712**
	Furans and Furanones						
G1	2,5-Dimethylfuran	953/946	n.d	n.d	n.d	n.d	27.91±0.68
G2	Furfural	1460/1479	29.16±0.81b	44.59±3.38a	22.35±0.82b	26.53±2.47b	39.3±0.51a
G3	2-Methyltetrahydrofuran-3-one	1243/1270	28.85±0.83b	33.23±3.16b	31.55±2.27b	36.11±2.2b	52.58±2.3ab

G4	2-Acetylfuran	1501/1483	n.d	n.d	n.d	n.d	15.2±0.08
G5	4-Hydroxy-2,5-dimethylfuran-3-one	2028/2039	127±8a	65.38±2.90b	33.28±2.97c	22.85±0.33c	24.96±0.94c
	Total		**185**	**143**	**87.18**	**85.49**	**160**
	Other heterocyclics						
H1	2-Acetylpyrrole	1972/1952	24.22±0.16c	27.9±1.57bc	40.59±1.34a	44.27±4.42a	34.68±2.34ab
H2	2-Piperidinone	2060/2060	68.15±3.15a	86.62±2.31a	n.d	n.d	17.39±1.41b
H3	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	2295/2266	n.d	n.d	n.d	n.d	35.35±3.27
	Total		**92.37**	**115**	**40.59**	**44.27**	**87.42**
	Others						
I1	Styrene	1250/1254	82.6±2.56a	78.67±6.32ab	53.39±4.87b	n.d	n.d
I2	Bicyclo[4.2.0]octa-1,3,5-triene	1272/1258	n.d	n.d	49.85±0.24b	60.73±5.78a	45.8±2.64b
I3	4-Ethyl-1,2-dimethylbenzene	1362/1379	n.d	n.d	n.d	n.d	122±3
	Total		**82.6**	**78.67**	**103**	**60.73**	**168**
	Total		**18549**	**18137**	**10812**	**16772**	**17770**

[a] Linear retention index on DB-Wax columns were determined by *n*-alkanes.

[b] Linear retention index on DB-WAX column from the literature. And "-" indicated the compound lack of reliable linear retention index value in literature

Table 6-3 The content of volatile compounds during reaction in pH 7.8 model.

Number	Compounds	LRI([a]/[b])	Contents（μg/kg）				
			2 h	4 h	6 h	8 h	10 h
	Alcohol						
A1	Isopropyl alcohol	912/935	n.d	8.47±0.11a	5.13±0.47b	n.d	n.d
A2	Ethanol	925/933	348±9a	62.26±3.97b	63.37±0.73b	57.72±3.94b	8.48±0.65c
A3	2-Methylpropan-1-ol	1119/1094	8.51±0.31b	14.95±0.5a	n.d	n.d	n.d
A4	3-Buten-1-ol	1178/1170	48.8±3.89	n.d	n.d	n.d	n.d
A5	2-Ethylhexanol	1483/1517	52.33±3.46a	20.92±1.5b	23.71±1.44b	17.35±0.33b	n.d
A6	Butane-2,3-diol	1554/1553	n.d	n.d	n.d	n.d	12.87±1.16
A7	Propylene glycol	1597/1603	n.d	n.d	n.d	n.d	22.34±0.76
	Total		**458**	**107**	**92.21**	**75.07**	**43.69**

	Aldehyde						
B1	2-Methylpentanal	1104/880	235±1a	130±7b	99.14±2.16c	223±12a	149±10b
B2	Methylglyoxal	970/-	24.11±0.64b	18.49±1.57c	32.65±2.11a	n.d	30.87±1.17a
	Total		**259**	**148**	**132**	**223**	**180**
	Ketone						
C1	Acetone	834/813	392±1b	256±13d	338±5c	1322±12a	79.38±3.52e
C2	4-Methyl-2-hexanone	850/-	n.d	8.53±0.69b	n.d	n.d	197±1a
C3	2-Hexanone	1100/1124	1277±75a	150±11d	434±21c	756±35b	282±6cd
C4	2-Heptanone	1174/1180	204±11b	57.21±3.17c	234±10b	340±25a	71.01±0.36c
C5	Hydroxyacetone	1275/1290	148±2a	93.86±7.89b	31.39±3.17c	15.56±0.57d	9.08±0.6d
C6	3-Hydroxybutan-2-one	1283/1286	358±1a	269±6c	284±4bc	340±29ab	346±7a
C7	2-Octanone	1294/1309	15181±213a	5925±33c	4396±8d	11632±13b	6056±158c
C8	2-Nonanone	1402/1386	61.68±2.03a	34.22±1.56b	34.43±0.11b	59.14±3.61a	n.d
C9	2-Decanone	1482/1493	300±2b	171±5c	143±1c	201±15c	398±39a
C10	2-Undecanone	1595/1599	15.3±0.31b	10.42±0.54c	n.d	n.d	34.83±1.12a
	Total		**17937**	**6975**	**5895**	**14666**	**7473**
	Acids						
D1	Acetic acid	1460/1429	68.73±1.66d	482±13a	382±9b	478.38±12.99a	325±20c
	Total		**68.73**	**482**	**382**	**478**	**325**
	Esters						
E1	Methyl pyruvate	1217/-	n.d	33.66±0.08b	36.75±1.13a	n.d	n.d
	Total		**n.d**	**33.66**	**36.75**	**n.d**	**n.d**
	Pyrazines						
F1	2-Methylpyrazine	1265/1274	34.66±2.51d	54.9±1.07bd	68.18±2.89b	89.51±5.9a	43.09±0.79cd
F2	2,5-Dimethylpyrazine	1321/1332	3633±58b	3715±246b	3887±221b	4490±178a	2964±172c
F3	2,3-Dimethylpyrazine	1342/1346	n.d	21.38±1.21	n.d	n.d	n.d
F4	2,6-Dimethylpyrazine	1348/1319	50.71±5.04c	60.17±0.79bc	71.25±2.09b	97.61±6.96a	76.04±0.87b
F5	2-Ethyl-6-methylpyrazine	1375/1363	38.3±0.21b	n.d	n.d	n.d	233±4a
F6	2-Ethyl-5-methyl-pyrazine	1378/1399	45.19±1.09d	91.69±5.01c	90.47±2.08c	143±13b	228±9a
F7	Trimethylpyrazine	1403/1413	77.92±2.29e	95.69±0.65d	111±1c	144±8b	161±2a
F8	3-Ethyl-2,5-dimethylpyrazine	1430/1455	n.d	n.d	n.d	n.d	21.87±1.86

F9	2-Ethyl-3,5-dimethylpyrazine	1464/1464	144±3c	129±6c	176±2c	254±22b	361±21a
F10	2-Allyl-5-methylpyrazine	1535/1535	n.d	n.d	n.d	n.d	22.21±2.21
F11	2-Methyl-6-(1-propenyl)pyrazine	1671/1539	n.d	n.d	n.d	n.d	22.43±1.7
F12	2-Acetyl-3-methylpyrazine	1629/1640	n.d	9.46±0.07	n.d	n.d	n.d
	Total		**4024**	**4177**	**4404**	**5218**	**4133**
	Furans and Furanones						
G1	2-Methyltetrahydrofuran-3-one	1243/1270	n.d	25.56±0.57b	31.39±1.17b	43.86±2.89a	22.08±0.6b
G2	Furfural	1460/1479	n.d	n.d	n.d	n.d	22.27±0.38
G3	2,5-Dimethylfuran	953/946	n.d	n.d	n.d	n.d	27.91±0.68
G4	2-Acetylfuran	1501/1483	n.d	n.d	n.d	n.d	6.28±0.12
G5	4-Hydroxy-2,5-dimethylfuran-3-one	2028/2039	n.d	n.d	n.d	16.35±1.47b	29.13±1.15a
	Total		**0**	**22.08**	**25.56**	**47.74**	**129**
	Other heterocyclics						
H1	2-Acetylpyridine	1587/1590	n.d	n.d	n.d	n.d	17.8±0.59
H2	2-Acetylpyrrole	1972/1952	n.d	n.d	n.d	n.d	109±2
H3	2-Piperidinone	2060/2060	17.96±0.61b	11.62±0.46b	48.76±4.78a	10.9±0.26b	n.d
	Total		**17.96**	**11.62**	**48.76**	**10.9**	**127**
	Others						
I1	Bicyclo[4.2.0]octa-1,3,5-triene	1272/1258	n.d	68.78±5.99a	65.09±1.69a	n.d	n.d
I2	2,4,6,8-Tetramethyl-1-undecene	1285/-	10.66±0.08	n.d	n.d	n.d	n.d
I3	Butylatedhydroxytoluene	1911/1920	190±13	n.d	n.d	n.d	n.d
	Total		**201**	**68.78**	**65.09**	**n.d**	**n.d**
	Total		**22965**	**12026**	**11081**	**20719**	**12411**

[a] Linear retention index on DB-Wax columns were determined by *n*-alkanes.

[b] Linear retention index on DB-WAX column from the literature. And "-" indicated the compound lack of reliable linear retention index value in literature

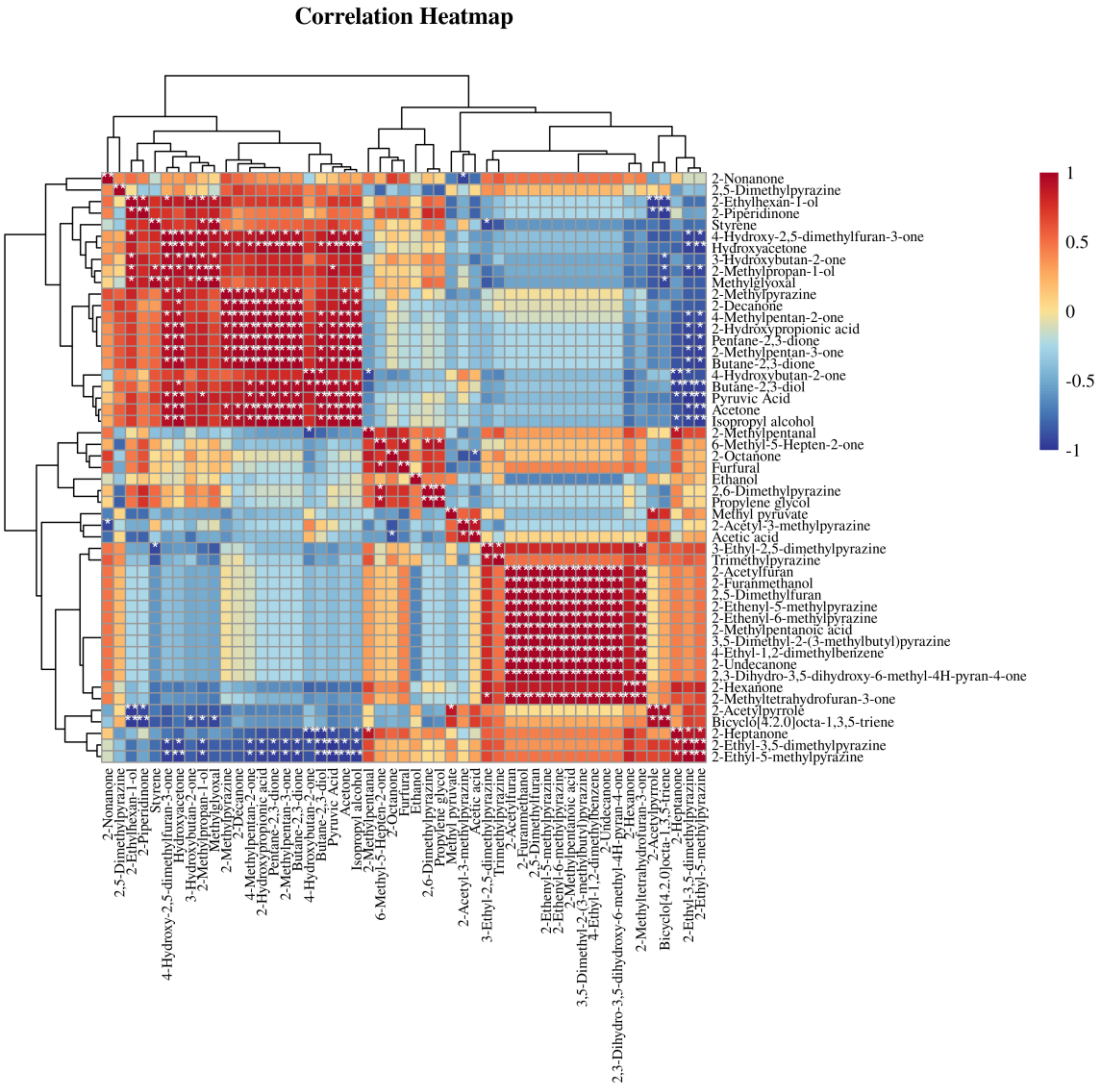
As typical intermediate products in the Maillard reaction, butane-2,3-diol, propylene glycol, methylglyoxal, acetone, butane-2,3-dione, pentane-2,3-dione, hydroxyacetone, 3-hydroxybutan-2-one and pyruvic acid mainly existed in the second and fourth hours of the reaction in the pH 5.5 model (**Table 6-2**). However, butane-2,3-diol, propylene glycol, methylglyoxal and butane-2,3-dione were present in higher concentrations in the sixth to the tenth hours during the reaction in the pH 7.8 model (**Table 6-3**). That was probably due to the fact that the reaction occurred faster in the pH 7.8 model, these intermediate products were consumed very quickly and there were not enough Lys to react in the late stage of the reaction, resulting in the accumulation of intermediate products.

3.3 Correlation analysis at different pH

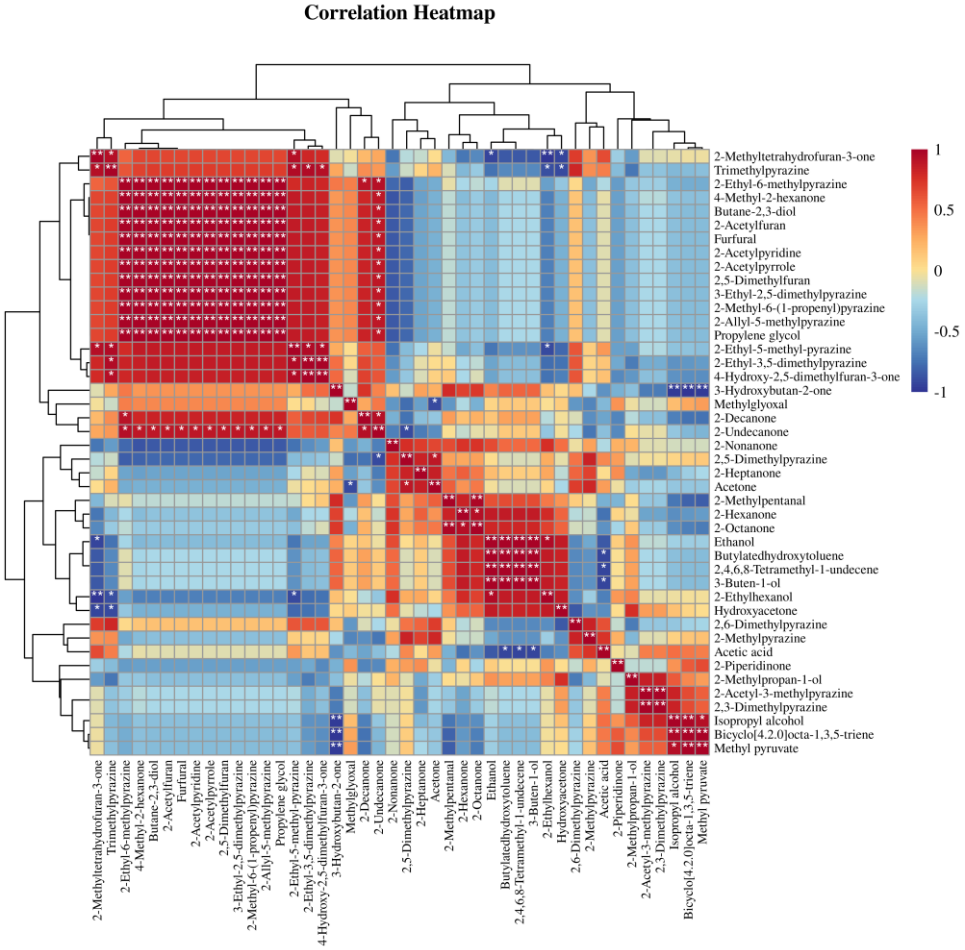
To further investigate the pyrazine formation process, the aroma compounds formed during the reaction were subjected to Pearson correlation analysis. From **Figure 6-1(a)** and **Figure 6-1(b)**, butane-2,3-diol, hydroxyacetone and propylene glycol showed a highly significant correlation with pyrazines ($p < 0.01$) in both pH 5.5 and pH 7.8 models. Butane-2,3-diol, as a precursor for pyrazines, is produced by the degradation of glucose, which can be further converted into an important α-dicarbonyl compound, namely butane-2,3-dione. Butane-2,3-dione can be transformed into 2-amino-3-butanone through Strecker degradation with amino acids and further form 2,3-dimethyl substituted pyrazines (Zhou et al., 2022). Similarly, propylene glycol, as a degradation product of glucose, can further form methylglyoxal and hydroxyacetone, both of which are also precursor compounds of pyrazines (Boekel, 2006; Jiang et al., 2022; Zhang et al., 2020). Hydroxyacetone can react with amino acids to form α-amino carbonyl compounds and then form pyrazines (A. N. Yu & Zhang, 2010).

In the pH 7.8 model, in addition to butane-2,3-diol, propylene glycol showed highly significant correlation with 2-allyl-5-methylpyrazine, 2-ethyl-6-methylpyrazine, 2-methyl-6-(1-propenyl)pyrazine and 3-ethyl-2,5-dimethylpyrazine ($p < 0.01$); hydroxyacetone showed a significant correlation with trimethylpyrazine and acetone showed a significant correlation with 2,5-dimethylpyrazine ($p < 0.05$). As an important intermediate product of Maillard reaction, 3-hydroxy-2-butanone only showed a significant correlation with hydroxyacetone and methylglyoxal ($p < 0.05$) in the pH 7.8 model (**Figure 6-1(b)**). 3-Hydroxy-2-butanone can be dehydrogenated to form butane-2,3-dione, which can form hydroxyacetone and

methylglyoxal, and provide the α-carbonyl compound for pyrazine formation.



(a) pH 5.5 model



(b) pH 7.8 model

Figure 6-1 Clustering correlation heatmap of volatile compounds during reaction process in pH 5.5 model (a) and pH 7.8 model (b)

In contrast to the pH 7.8 model, in addition to butane-2,3-diol, hydroxyacetone and propylene glycol were significantly correlated with pyrazines, and butane-2,3-dione showed a significant correlation with 2-ethyl-5-methylpyrazine, 2-methylpyrazine and 3,5-EDMP ($p < 0.05$) in the pH 5.5 model (**Figure 6-1(a)**). Butane-2,3-dione can form acetaldehyde, glyoxal and methylglyoxal via thermal degradation, and these intermediates can form aminoacetone, 2-aminoacetone and 2-aminobutan-3-one by Strecker degradation with amino acids, which in turn can be

condensed to form pyrazines (Guerra & Yaylayan, 2012). Pentane-2,3-dione was also significantly correlated with 2-ethyl-5-methylpyrazine, 2-methylpyrazine and 3,5-EDMP ($p < 0.05$) in the pH 5.5 model (**Figure 6-1(a)**). Pentane-2,3-dione is a precursor of 3,5-EDMP and 2-ethyl-3,6-dimethylpyrazine (3,6-EDMP), which can produce 2-aminopentan-3-one and 3-aminopentan-2-one via Strecker degradation, which are subsequently condensed with aminoacetone to produce the corresponding pyrazines (Zhang et al., 2020). Pyruvic acid had a highly significant correlation with 2-ethyl-5-methylpyrazine and 3,5-EDMP ($p < 0.01$) and significant correlation with pentane-2,3-dione ($p < 0.05$) in the pH 5.5 model (**Figure 6-1(a)**). Pyruvic acid is also an important intermediate in the formation of pyrazine, which can be produced from methylglyoxal and then further reacts to form 2-hydroxypropionic acid and finally 2,3-pentanedione, providing a rich precursor for pyrazine formation (Albouchi & Murkovic, 2020; Glomb, 2017). Besides, isopropyl alcohol, acetone and 2-hydroxypropionic acid also had a significant correlation with 2-ethyl-5-methylpyrazine, 2-methylpyrazine and 3,5-EDMP ($p < 0.05$) in the pH 5.5 model (**Figure 6-1(a)**). The above-mentioned intermediate products of the Maillard reaction not only showed significant correlations with pyrazines but also had significant correlations between them. For example, 3-hydroxybutan-2-one had a highly significant correlation ($p < 0.01$) with isopropyl alcohol, acetone had a significant correlation with methylglyoxal ($p < 0.05$) and butane-2,3-diol had a highly significant correlation with propylene glycol ($p < 0.01$) at pH 7.8. At pH 5.5, there were more correlations observed between the intermediate products of the Maillard reaction, such as between 3-hydroxybutan-2-one, hydroxyacetone, methylglyoxal, butane-2,3-diol, pyruvic acid, isopropyl alcohol, butane-2,3-dione, 2-hydroxypropionic acid, pentane-2,3-dione and acetone. These correlations could provide ideas for the formation of pyrazines in red jujube.

3.4 Freeze drying verification experiment based on real system of red jujube

In order to further validate the formation of pyrazine during freeze drying of red jujube, the odourless matrix, model 3 (Lys + Glu + odourless matrix) and model 4 (Lys + Glu + red jujube) were established, and then the three samples and red jujube were freeze dried under industrial conditions. From **Table 6-4**, model 4 showed the highest content of total aroma compounds (27,123 μg/kg) among all samples after freeze drying. Especially, ketones (11,309 μg/kg) and pyrazines (6,796 μg/kg) were

the most abundant compounds in model 4, and the content of both was much higher than that of model 3 and red jujube after freeze drying. This result suggested that the aroma of processed red jujube products can be modulated by adding relevant aroma precursors, such as amino acids. Since red jujube are richer in sugars, there is more pyrazine production after the addition of amino acids due to the occurrence of the Maillard reaction.

As the typical intermediate products of the Maillard reaction, methylglyoxal, butane-2,3-dione and pentane-2,3-dione were detected only in model 2, indicating that the Maillard reaction in model 2 was sufficiently carried out, and the amino acids were fully utilised. 3-Hydroxybutan-2-one and acetic acid are abundant compounds in red jujube (Gou et al., 2022); therefore, they still had a higher content in freeze-dried red jujube compared to freeze-dried model 3 and 4. As another abundant compound in red jujube (Gou et al., 2022), the formation of lactones involves fatty acid metabolism (Wan et al., 2012), but only the Maillard reaction occurred in model 3, and lactones were not detected in model 3. Although less lactone was detected in model 4, it may be because the addition of amino acids resulted in the Maillard reaction becoming the dominant reaction, which in turn blocked the formation of lactones in model 4.

Comparison of the aroma compounds at the end of the reaction in model 1 with the aroma compounds after freeze drying in model 3 (**Table 6-2 and Table 6-4**) showed that the total content of aroma compounds in model 3 (9,950 μg/kg) was lower than that in model 1 (17,790 μg/kg), and there was a difference in the type of pyrazines, but the difference in the content of pyrazine was not significant (3,776 μg/kg in model 3 and 3,712 μg/kg in model 1). Besides, in both model 1 and model 3, 2-methylpentanal was the only detected aldehyde, acetic acid was the major acid, 2-hexanone and 2-octanone were the dominant ketones, DDMP (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one) was the major heterocyclic compound and 2,5-dimethylpyrazine was the major pyrazine, and these results indicated that model 1 can better simulate the formation of aroma under freeze-drying conditions. Therefore, the pathway of pyrazine formation in red jujube during freeze drying was predicted by combining the correlation analysis of Model 1 and the aroma compounds generated in Model 4.

Table 6-4 The content of volatile compounds in the odourless matrix, model 3 (Lys + Glu + odourless matrix) and model 4 (Lys + Glu + red jujube) under the freeze drying condition.

Numbers	Compounds	LRI([a]/[b])	Contents (μg/kg)			
			Odourless matrix	Model 3	Red jujube	Model 4
	Alcohol					
A1	Propan-2-ol	912/920	n.d	n.d	n.d	12.67±0.69
A2	Oct-1-en-3-ol	1450/1450	n.d	n.d	141±12b	877±21a
A3	Butane-2,3-diol	1554/1553	n.d	n.d	186±4a	97.67±8.57b
	Total		**0**	**0**	**327**	**987**
	Aldehyde					
B1	2-Methylpentanal	725/-	n.d	89.56±4.46	n.d	n.d
B2	Methylglyoxal	970/-	n.d	n.d	n.d	46.63±1.6
B3	Hexanal	1083/1078	n.d	n.d	56.51±4.21	n.d
B4	2-Hexenal	1248/1216	n.d	n.d	n.d	n.d
B5	2-Heptenal	1336/1318	n.d	n.d	n.d	n.d
B6	2-Octenal	1429/1410	n.d	n.d	n.d	n.d
B7	2,2-Dimethylhexanal	1452/1452	41.91±0.04	n.d	n.d	n.d
B8	Benzaldehyde	1480/1508	51.14±1.59b	n.d	81.71±5.88a	41.38±0.87b
	Total		**93.05**	**89.56**	**138**	**88.01**
	Ketone					
C1	4-Methylhexan-2-one	850/-	n.d	n.d	n.d	29.90±0.22
C2	Butane-2,3-dione	964/979	n.d	n.d	n.d	46.14±0.64
C3	4-Methylpentan-2-one	1006/1028	n.d	n.d	n.d	1090±100
C4	Pentane-2,3-dione	1055/1062	n.d	n.d	n.d	824±35
C5	2-Hexanone	1100/1124	n.d	1109±26	n.d	n.d
C6	2-Heptanone	1174/1180	n.d	159±10	n.d	n.d
C7	3-Octanone	1253/1240	n.d	n.d	10.89±0.81	n.d
C8	3-Hydroxybutan-2-one	1283/1286	n.d	n.d	471±20a	17.96±0.29c
C9	2-Octanone	1294/1309	n.d	4667±318b	n.d	8946±228a
C10	6-Methyl-5-hepten-2-one	1365/1342	n.d	11.42±0.63c	84.64±0.09a	52.12±0.95b

C11	3-Acetoxy-2-butanone	1378/1361	n.d	n.d	35.91±3.55	n.d
C12	2-Decanone	1482/1493	n.d	n.d	n.d	262±6
C13	4,5-Dimethyloxolan-2-one	1590/-	n.d	n.d	74.16±2.12	n.d
C14	6,10-Dimethylundecan-2-one	1660/-	n.d	n.d	114±8	n.d
C15	3,5-Dimethyloxolan-2-one	1671/-	n.d	n.d	26.47±2.71a	13.04±0.25b
	Total		**0**	**5946**	**817**	**11281**
	Acids					
D1	Pyruvic Acid	1249/-	n.d	30.32±1.35	n.d	n.d
D2	Acetic acid	1460/1429	98.85±8.01b	33.93±3b	585±215a	n.d
D3	Propanoic acid	1557/1508	n.d	n.d	282±1	n.d
D4	Butanoic acid	1612/1628	49.93±0.58b	n.d	283±11a	n.d
D5	3-Methylbutanoic acid	1666/1680	n.d	n.d	213±17a	94.98±4.96b
D6	Pentanoic acid	1733/1762	n.d	n.d	112±10a	67.14±0.14b
D7	(*E*)-But-2-enoic acid	1745/1750	n.d	n.d	22.68±8.32	n.d
D8	Hexanoic acid	1846/1849	n.d	n.d	249±1b	294±19a
D9	Heptanoic acid	1950/1943	n.d	n.d	174±4a	118±10b
D10	2-Hexenoic acid	1967/1994	n.d	n.d	17.71±1.35	n.d
D11	Octanoic acid	2060/2086	n.d	n.d	181±2	n.d
D12	Nonanoic acid	2169/2171	n.d	n.d	32.18±0.65	n.d
D13	*n*-Decanoic acid	2289/2270	n.d	n.d	90.61±2.83a	69.65±6.57b
	Total		**149**	**64.25**	**2242**	**644**
	Esters					
E1	Methyl acetate	828/810	n.d	n.d	n.d	151±5
E2	Methyl hexanoate	1184/1177	n.d	n.d	142±7b	405±22a
E3	Ethyl hexanoate	1231/1241	n.d	n.d	424±27a	142±7b
E4	Hexyl acetate	1272/1265	n.d	n.d	8.18±0.47	n.d
E5	Ethyl heptanoate	1342/1342	n.d	n.d	144±6	n.d
E6	Methyl octanoate	1380/1374	n.d	n.d	230±20b	251±5a
E7	Ethyl octanoate	1452/1441	n.d	n.d	273±19a	195±5b
E8	Methyl nonanoate	1481/1536	n.d	n.d	91.29±5.08	n.d

E9	Methyl decanoate	1593/1636	n.d	n.d	1292±113b	1833±26a
E10	Ethyl decanoate	1638/1633	n.d	n.d	2453±162a	840±1b
E11	Methyl undecanoate	1703/1732	n.d	n.d	39.23±0.62	n.d
E12	Methyl dodecanoate	1804/1834	176±8c	n.d	1644±62b	1957±49a
E13	Ethyl dodecanoate	1841/1849	n.d	n.d	1801±52a	663±13b
E14	Methyl myristoleate	2000/2000	n.d	n.d	673±28a	136±2b
E15	Methyl hexadecanoate	2208/2243	n.d	n.d	70.66±2.12	n.d
	Total		**176**	**0**	**9285**	**6573**
	Lactones					
F1	Butyrolactone	1665/1643	n.d	n.d	27.71±1.94	n.d
F2	5-Ethyloxolan-2-one	1694/1736	n.d	n.d	68.63±0.03b	71.41±0.5a
F3	6-Methyloxan-2-one	1772/1751	n.d	n.d	45.99±3.47	n.d
F4	5-Propyloxolan-2- one	1787/1796	n.d	n.d	20.16±0.61	n.d
F5	5-Butyloxolan-2-one	1910/1936	n.d	n.d	32.68±0.79	n.d
F6	5-Heptyloxolan-2- one	2024/2247	n.d	n.d	4.58±0.83	n.d
F7	5-Hexyloxolan-2-one	2177/2113	n.d	n.d	5.73±0.37	n.d
	Total		**0**	**0**	**205**	**71.41**
	Pyrazines					
G1	2-Methylpyrazine	1265/1274	n.d	n.d	n.d	46.42±0.02a
G2	2,5-Dimethylpyrazine	1321/1332	n.d	3581±136b	n.d	5761±250a
G3	2,6-Dimethylpyrazine	1348/1319	n.d	31.87±1.07b	6.49±0.79c	149.01±4.25a
G4	2-Ethyl-6-methylpyrazine	1375/1363	n.d	n.d	47.06±3.40	n.d
G5	2-Ethyl-5-methylpyrazine	1378/1399	n.d	77.46±1.02b	n.d	215±19a
G6	Trimethylpyrazine	1403/1413	n.d	32.47±2.91b	n.d	158±1a
G7	2-Ethyl-3,5-dimethylpyrazine	1455/1464	n.d	53.45±3.42b	22.8±0.90b	372±9a
G8	2-Allyl-5-methylpyrazine	1535/1535	n.d	n.d	n.d	94.35±4.19
	Total		**0**	**3776**	**76.35**	**6796**
	Furans					
H1	2-Methyloxolan-3-one	1243/1270	n.d	n.d	n.d	27.35±2.42
H2	Furfural	1460/1479	n.d	39.87±3.26c	68.58±0.43b	101±8a

	Total		**0**	**39.87**	**68.58**	**128**
	Other heterocyclics					
I1	2-Acetylpyrrole	1972/1952	n.d	13.25±0.89b	n.d	27.65±0.67a
I2	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	2295/2266	n.d	20.76±1.73b	n.d	89.65±1.48a
	Total		**0**	**34.01**	**0**	**117**
	Others					
J1	2,2-Dimethylbutane	536/500	108±2	n.d	n.d	n.d
J2	2,4-Dimethylpentane	611/-	n.d	n.d	n.d	n.d
J3	2,4-Dimethylhexane	735/-	280±13	n.d	n.d	n.d
J4	Limonene	1200/1189	n.d	n.d	433±41a	220±1b
J5	.gamma.-Terpinene	1246/1238	n.d	n.d	7.03±0.53	n.d
J6	Styrene	1250/1254	n.d	n.d	121±8b	217±16a
J7	*p*-Cymene	1270/1261	n.d	n.d	12.69±0.16	n.d
J8	.alpha.-Farnesene	1746/1754	n.d	n.d	26.79±0.21	n.d
J9	Naphthalene	1746/1707	n.d	n.d	24.83±1.62	n.d
	Total		**388**	**0**	**625**	**437**
	Total		**806**	**9950**	**13786**	**27123**

[a] Linear retention index on DB-Wax columns were determined by *n*-alkanes.

[b] Linear retention index on DB-WAX column from the literature. And "-" indicated the compound lack of reliable linear retention index value in literature

3.5 Predicted formation pathway of alkylpyrazines based on red jujube matrix

Red jujube is rich in amino acids, glucose, fructose and sucrose, which are important substrates for the Maillard reaction (Gou, et al., 2023). During thermal processing, 3-hydroxybutan-2-one, 2-hydroxypropionic acid, hydroxyacetone, butane-2,3-diol and pyruvic acid can be formed from degradation of these sugars (Zhang et al., 2020; Zhou et al., 2022). Then, some α-dicarbonyl compounds, such as butane-2,3-dione, pentane-2,3-dione, methylglyoxal, acetaldehyde and glyoxal, can be generated from the above-mentioned products. These α-dicarbonyl compounds can be converted to α-aminocarbonyl compounds via a Strecker degradation reaction with α-amino acids, such as aminoethanone (A), aminoacetone (B), 2-Aminoacetone (C), 3-amino-butan-2-one (D), 2-aminopentan-3-one (E) and 3-aminopentan-2-one (F) (Guerra & Yaylayan, 2012; Zhang et al., 2020). Next, dihydropyrazine will be formed through the condensation of two α-aminocarbonyl compounds. Furthermore, the formation of pyrazines will involve two pathways, one is the spontaneous conversion of dihydropyrazine to alkylpyrazines through oxidation. The other pathway is deprotonation of dihydropyrazine and reaction with aldehyde compounds to form alkylpyrazines via an aldol-type reaction (Scalone et al., 2015). These aldehyde compounds can also be generated from Strecker degradation of amino acids or thermal degradation from sugars (Mei et al., 2007).

In all models, 2,5-dimethylpyrazine showed a higher content than 2,6-dimethylpyrazine; since aldehydes are typically more reactive than ketones, the 2,5-configuration is preferred over the 2,6-configuration (Mei et al., 2007). As the key aroma compound in freeze-dried red jujube, 3,5-EDMP also can be formed via two pathways. One is by Strecker degradation of pentane-2,3-dione, which yields 2-aminopentan-3-one (E), which can be directly condensed with aminoacetone (B) obtained from Strecker degradation of methylglyoxal to form 3,5-EDMP (Pathway A (B+E), **Figure 6-2**). Another pathway is the condensation of aminoacetone (B) and 2-aminoacetone (C) from Strecker degradation of methylglyoxal to form 2,6-dihydropyrazine. Then 2,6-dihydropyrazine is deprotonated and reacted with acetaldehyde (G) from amino acid Strecker degradation or from sugar degradation to produce 3,5-EDMP (Pathway B (B+C+F), **Figure 6-2**). In general, 3,6-EDMP shares a similar pathway with 3,5-EDMP. One pathway is the result of condensation from 2-aminoacetone from methylglyoxal and 3-aminopentan-2-one from pentane-2,3-

dione (Pathway A (C+F), **Figure 6-2**), and another is the condensation of two aminoacetone to form 2,5-dihydropyrazine. Then 2,5-dihydropyrazine is deprotonated and reacted with acetaldehyde to produce 3,6-EDMP (Pathway B (B+B+F), **Figure 6-2**). The amination of R-ketoaldehydes occurs favourably at the aldehydic position, so methylglyoxal is more likely to produce aminoacetone (B) than 2-aminoacrtone (C) through Strecker degradation (Mei et al., 2007). However, 3,6-EDMP was barely detected in all samples, suggesting that Pathway A may be the dominant pathway for EDMP formation in the red jujube matrix. Besides, in Pathway A, 2,3-pentanedione preferentially generates 2-aminopentan-3-one (E) over 3-aminopentan-2-one (F), resulting in a higher level of 3,5-EDMP than 3,6-EDMP in red jujube matrix.

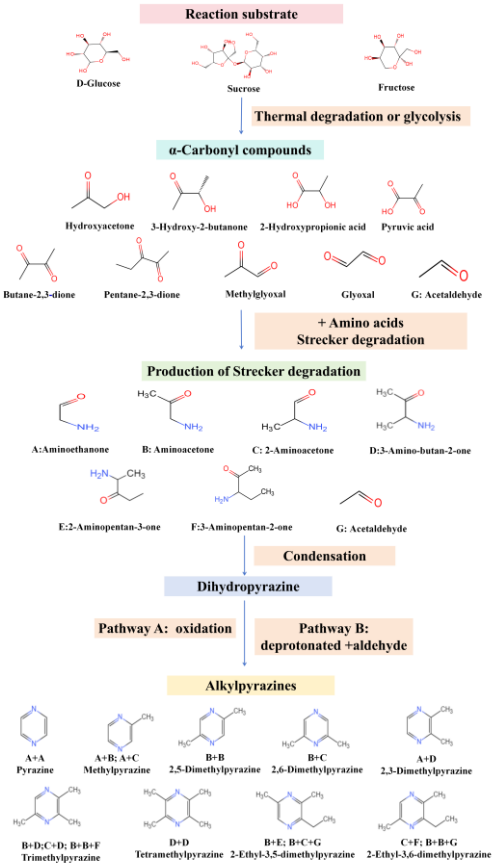


Figure 6-2 Formation pathway of alkylpyrazines in red jujube.

4. Conclusion

In this study, the pathway of alkylpyrazine formation was investigated based on the red jujube matrix. The total content of volatile compounds in the pH 5.5 model was greater than that in the pH 7.8 model, while the content of pyrazines was lower than pH 7.8. The reaction occurred much faster in the pH 7.8 model. Through correlation analysis, butane-2,3-diol, hydroxyacetone and propylene glycol showed a highly significant correlation with pyrazines in both pH 5.5 and pH 7.8 models. Besides, butane-2,3-dione, pentane-2,3-dione, pyruvic acid, isopropyl alcohol, acetone and 2-hydroxypropionic acid were significantly correlated with 3,5-EDMP in the pH 5.5 model. The formation pathway of 3,5-EDMP in red jujube matrix prefers direct condensation from aminoacetone and 2-aminopetan-3-one (Pathway A).

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Chapter VII. Characterization of key aroma-active compounds changes and formation in red jujube as affected by different processing methods via GC-MS and untargeted metabolomics

In addition to freeze drying, common processing methods for red jujube include steaming, frying and baking. Different processing methods presented significant effect on the aroma of red jujube. Therefore, the aim of this chapter was to investigate the effect of steaming, freeze drying, frying and baking on the aroma of red jujube. Furthermore, the formation pathway of key aroma compounds in different processing methods was explored.

Gou, M., Bi, J., Liu, G., Fauconnier, M.-L., & Chen, Q. (2023). Characterization of key aroma-active compounds changes and formation in red jujube as affected by different processing methods via GC-MS and untargeted. Revised.

Abstract: Different processings presented significant effect on the aroma of red jujube, however, few studies have investigated how processing affected the aromas. The changes in aroma of red jujube after freeze drying, baking, frying and steaming were investigated by GC-MS, GC-O, OAV and quantitative descriptive analysis. After freeze drying, the total aroma content increased by 0.90%, while it decreased by 51.59%, 74.11% and 78.74% after baking, frying and steaming, respectively. Furthermore, the formation of aroma-active compounds via different processings was predicted based on untargeted metabolomics. Esters dominated the aroma and contributed sweet and fruity notes in freeze-dried jujubes, especially ethyl esters, which were formed via combination of fatty acids metabolism, pyruvate metabolism and amino acid catabolism. Aldehydes, especially (*E, E*)-deca-2,4-dienal dominated the fatty note in fried samples, which were the products of lipid oxidation and fatty acid metabolism. Pyrazines dominated the roasty notes of baked samples due to the Maillard reaction.

Keywords: freeze drying, frying, steaming, baking, untargeted metabolomics

1.Introduction

As a kind of traditional medicine and edible fruit in China, red jujube (*Ziziphus jujuba* Mill.) is popular owing to its pleasant flavour and high nutritional value (Gou et al., 2022). In daily life, red jujube is usually eaten fresh or cooked by steaming or boiling. Consumers prefer processed red jujube products, such as jujube crisps, jujube juice, jujube cake, powdered jujube, and fried jujube, to the raw form. Traditional heat processing methods of red jujube include hot air drying, frying and others. In comparison with other drying techniques, freeze drying (FD) was superior for retaining the colour, shape, texture and nutritional value of red jujubes, and was increasingly prevalent in the manufacture of leisure food. In addition, red jujube is extensively used as an ingredient in flavouring and cake filling.

Aroma is one of the important quality of red jujube products, which contain alcohols, aldehydes, ketones, acids, pyrazines, alkenes, lactones, and esters (Gou et al., 2022; Zhu & Xiao, 2018). Also, there are abundant aroma precursors in red jujube, such as glucose, fructose, sucrose, amino acids and fatty acids, that can provide substrates for the formation of aroma (Gou, et al., 2023). Due to the influence of different temperatures, heat transfer methods and heating media in the process, red jujube will undergo Maillard reactions, lipid oxidation, amino acid degradation, and their combined reactions, resulting in significant differences in the aroma and quality of the products (Wei et al., 2023; Zhou et al., 2022). However, information on how processing methods potentially affect the aroma quality of red jujube was still insufficient.

Volatile compounds with a variety of odours determine the aroma characteristics of red jujube, that could be analysed based on extraction and qualified methods. In most studies, headspace-solid phase microextraction (HS-SPME) was used (Liu et al., 2021). Validated methods for aroma compounds mainly include gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O) (Gou et al., 2022; Zhu & Xiao, 2018), and gas chromatography-ion mobility spectrometry (GC- IMS) (Qiao et al., 2021; Yang et al., 2019). Moreover, GC coupled with tandem MS (GC-MS/MS) could further decrease detection limits in targeted food analysis and provide the sensitivity and selectivity required of the sample (Hopfer et al., 2016). In addition, metabolomics technology could be used to identify amino acids, organic acids, fatty acids and sugars, which are precursors of volatile compounds, by untargeted or targeted comprehensive metabolite analysis

based on spectrometry (MS) or non-MS techniques (Fu et al., 2022). It is also to predict potential metabolic pathways based on the conversion of metabolites, which is an efficient way to characterize the variation in volatile compounds in red jujube during processing.

Therefore, the purpose of this study was to investigate the effect of typical processing methods, including steaming, freeze drying, frying and baking, on the aroma of red jujube by quantitative descriptive analysis, GC-O, GC-MS and OAV. Furthermore, the formation pathway of key aroma compounds in different processing methods was explored via untargeted metabolomics.

2.Materials and methods

2.1. Materials and chemicals

All red jujube (*Zizyphus jujuba* cv. Huizao) were collected from Akesu, the Xinjiang Uyghur Autonomous Region, China. Red jujubes without mechanical damage were stored in the laboratory and immediately stored at 4 ^{o}C until use.

Butane-2,3-diol (≥ 98%), oct-1-en-3-ol (≥ 98%), 3-methylbutanal (≥ 98%), 2-propylfuran (≥ 98%), hexanal (≥ 98%), (E)-hex-2-enal (≥ 98%), 2-pentylfuran (≥ 98%), octanal (≥ 98%), (E)-hept-2-enal (≥ 98%), nonanal (≥ 98%), (*E*)-oct-2-enal (≥ 98%), furfural (≥ 98%), benzaldehyde (≥ 98%), decanal (≥ 98%), butane-2,3-dione (≥ 98%), pentane-2,3-dione (≥ 98%), 3-hydroxybutan-2-one (≥ 98%), 6-methylhept-5-en-2-one (≥ 98%), 3-oxobutan-2-yl acetate (≥ 98%), acetic acid (≥ 98%), propionic acid (≥ 98%), butanoic acid (≥ 98%), 3-methylbutanoic acid (≥ 98%), pentanoic acid (≥ 98%), (*E*)-but-2-enoic acid (≥ 98%), hexanoic acid (≥ 98%), heptanoic acid (≥ 98%), nonanoic acid (≥ 98%), decanoic acid (≥ 98%), dodecanoic acid (≥ 98%), methyl acetate (≥ 98%), methyl hexanoate (≥ 98%), ethyl hexanoate (≥ 98%), hexyl acetate (≥ 98%), ethyl heptanoate (≥ 98%), methyl octanoate (≥ 98%), ethyl octanoate (≥ 98%), methyl nonanoate (≥ 98%), methyl decanoate (≥ 98%), methyl benzoate (≥ 98%), ethyl decanoate (≥ 98%), methyl dodecanoate (≥ 98%), ethyl dodecanoate (≥ 98%), methyl tetradecanoate (≥ 98%), methyl hexadecanoate (≥ 98%), oxolan-2-one (≥ 98%), 5-ethyloxolan-2-one (≥ 98%), 5-propyloxolan-2-one (≥ 98%), 5-butyloxolan-2-one (≥ 98%), 5-heptyloxolan-2-one (≥ 98%), 2-ethyl-5-methylpyrazine (≥ 98%), 2-ethyl-6-methylpyrazine (≥ 98%), trimethylpyrazine (≥ 98%), 2,6-diethylpyrazine (≥ 98%), 2-ethyl-3,5-dimethyl-pyrazine (3,5-EDMP) (≥ 98%), tetramethylpyrazine (≥ 98%), limonene (≥ 98%), γ-

terpinene (≥ 98%), styrene (≥ 98%), *p*-cymene (≥ 98%), 1,4-xylene (≥ 98%), naphthalene (≥ 98%), 2-cyclohexene-1-one (≥ 98%), *n*-alkane (C5-C40) were obtained from Yuanye Bio-Technology (Shanghai Yuanye Bio-Technology Co., Ltd, Shanghai, China) and Macklin (Shanghai Macklin Biochemical Co., Ltd, Shanghai, China).

2.2. Sample preparation

Following cleaning and removal of the kernel, red jujube samples were cut into 5 mm-thick slices and processed by freeze drying, baking, frying or steaming. Steamed red jujbe in a covered steamer for 10 minutes. For frying, red jujube were put in corn oil preheated to 210 °C for 10 seconds. For baking, red jujube were put on the baking pan of an oven (PT 2531, Media Co., Ltd., Anhui, China) at 180 °C for 5 min. For freeze-dried red jujube, the freeze drying conditions are described in Gou et al., (2023).

2.3. Sensory evaluation

Sensory evaluation method is described in Gou et al., (2021) and Gou et al., (2023). Briefly, 10 panellists with experience in quantitative descriptive analysis (QDA) formed the sensory panel. In order to evaluate the odour description (creamy, fatty, green, fruity, roasty, sweet, sour, nutty and caramel notes) and intensity, standard solutions of 3-hydroxybutan-2-one, (*E*, *E*)-2, 4-decadienal, hexanal, methyl dodecanoate, 2,6-dimethylpyrazine, (*E*)-but-2-enoic acid, acetic acid, 2-ethyl-6-methylpyrazine and furaneol were dissolved in water (containing 5% methanol) and final diluted to 100 times of its odour threshold. These samples were put in 20 mL vials and randomly numbered. Panellists identified the odour and score the intensity from 0 to 9 in increments of 1 (0, none and 9, very strong).

2.4. Headspace solid-phase microextraction (HS-SPME)

A 65 μm polydimethylsiloxane-divinylbenzene (PDMS/DVB/CAR) fiber were used in this study. The extraction conditions for volatile components of different samples by HS-SPME were described by Gou, et al., (2023) and Gou et al., (2022).

2.5. GC-MS analysis

The GC-MS/MS (Trace 1300 GC system, TSQ 9000 MS/MS, Thermo Fisher Scientific, U.S.) equipped with a DB-Wax column (30 m × 0.25 mm, 0.25 μm, Agilent, U.S.) was used to analyse aroma compounds. The carrier gas was Helium

(99.999%) at a flow rate of 1.0 mL/min. The oven temperature started at 40 °C for 3 min, was raised to 120 °C at 5 °C/min, and was finally heated to 200 °C at 10 °C/min, and held for 5 min. Only one MS was used to identify the compounds. And the mass spectrometer was Electron-impact mode (EI), fragments were collected in scan mode from 35 to 500 m/z. The qualitative analysis using NIST17 library, retention indices (RI) and aroma standards. And the quantitative analysis using internal standard (2 μL 2-cyclohexene-1-one, 1 mg/L).

2.6. GC-O-MS analysis

A sniffing port (OP275 Pro II, GI Sciences, Japan) coupled to a GC-MS was applied to identify the aroma-active compounds. The GC effluent was split in a ratio of 7:3 between an olfactometric port and the MS detector. The temperature of sniffing port was 120 °C (Gou, et al., 2023).

2.7. Odour activity value (OAV)

The OAV was determined as follows:

OAV=C/OT

where: C presented the concentration of the compound, and OT presented its threshold in water. The threshold values of different components presented referenced in the literature (Gemert, 2011; Jia et al., 2019; Wei et al., 2023).

2.8. Analysis of HPLC-MS/MS Metabolite

Metabolite extraction: the samples were frozen by liquid nitrogen and ground into a powder with a Joyoung pulveriser (JYL-CO20, Joyoung Co., Ltd., Shandong, China). And, put 100 mg of powder into 500 μL lysate (MeOH: H2O = 1:1), vortexed for 30 s, and kept at -20 °C overnight. Then centrifuged at 13,000 rpm for 20 min at 4 °C, collected the supernatant, and 100 μL was drawn into an injection bottle.

In order to better collect data and ensure the best condition of the instrument, the chromatographic column is balanced before the formal sample is loaded on the machine. A quality control sample (QC) is inserted in the middle of every 10 samples to ensure the stability of the instrument throughout the running batch.

HPLC conditions: the metabolites of different processed red jujube were analysed by an HPLC system (ExionLC, AB SCIEX, Redwood City, CA, USA) with a Waters HSS T3 column (4.6 mm × 150 mm, 3.5 μm). The mobile phase were in water

(containing 0.1% formic acid) (A) and acetonitrile (0.1% formic acid) (B). The program of gradient was 99% A, 0 -0.5 min; 99% -50% A, 0.5 -2 min; 50% -1% A, 2 -9 min; 1% A, 9 -10 min; 1% -99% A, 10 -10.5 min; 99% A, 10.5 -14 min. The column temperature was 40 oC, and the elution gradient was 0.3 mL/min.

Mass conditions: MS/MS spectra was acquired with a TripleTOF 5600+ mass spectrometer (AB SCIEX, Redwood City, CA, USA). Data acquisition used the information-dependent acquisition (IDA) high-sensitivity scanning mode of the Analyst 1.6 software (AB SCIEX, Redwood City, CA, USA). Both positive- and negative-ionization models were used. The ion source parameters were set as follows: sheath gas flow rate, 30 Arb; auxiliary gas flow rate, 55 Arb; sweep gas flow rate, 55 Arb; temperature, 550 °C; spray voltage, 5.5 kV (positive) or -4.5 kV (negative). In full-scan TOF-MS, both the TOFMS and the MS/MS range were set at 50 -1200 m/z.

The original data were obtained on the basis of mass spectrometry detection, and were imported into ProgenesisQI (Waters) software for peak identification, extraction, alignment and integration. The final dataset contained the m/z, RT, peak number, sample name, normalized peak area and intensity of all detected ions. Then, the above-mentioned QC analysis was conducted to ensure the accuracy and reliability of the data. Metabolites with CV < 30%, VIP > 1 and P < 0.05 in QC samples were chosen as differential metabolites for further analysis. Moreover, the KEGG (https://www.genome.jp/kegg/), ChemSpider (https://www.chemspider.com/), PubChem (https://pubchem.ncbi.nlm.nih.gov/) and HPlantCyc (https://plantcyc.org/) databases were used in this study.

2.9. Statistical analysis

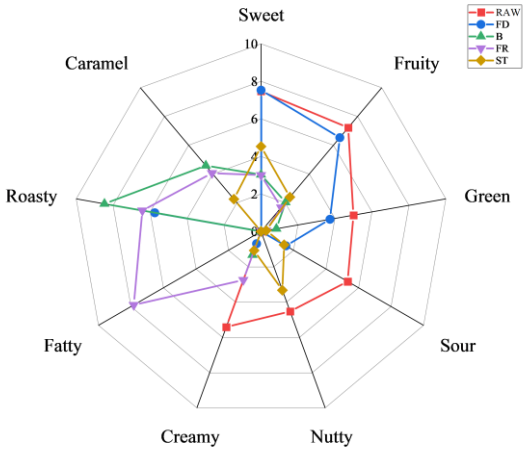
The software SPSS version 20.0 (Armonk, NY: IBM Corp.) was performed for statistical analysis. Significant differences were presented by Duncan's test ($p < 0.05$). Results were performed by mean ± standard deviation. The heatmap was created by TBtools version 1.0686. The Venn diagram was plotted using http://bioinformatics.psb.ugent.be/webtools/Venn/. The Sankey diagram was plotted in Origin and the volatile compound network was plotted by Cytoscape version 3.8.2.

3.Results and discussion

3.1. Sensory evaluation of red jujube processed by different

methods

The aroma profiles of samples after different processed were evaluated through quantitative descriptive sensory analysis (QDA), and the results are displayed in **Figure 7-1(a)**. There were nine sensory attributes to describe the aroma of all samples, involving sweet, fruity, green, fatty, roasty, creamy, sour, nutty and caramel notes. From **Figure 7-1(a)**, the intensity of these aroma characteristics showed significant differences ($p < 0.05$) among five samples. For raw red jujube, the aroma profile related to sweet, fruity, green, creamy, sour and nutty notes, that was line in our previous study (Gou et al., 2022). Steamed samples had an increase in caramel notes and fried samples had increased fatty, roasty and caramel notes. The baked samples also presented strong roasty and caramel notes. In both fried and baked samples, sour notes were imperceptible, and there was a sharp decrease in sweet, fruity, green and nutty notes, which might have a correlation with the reduction of acids, esters and ketones (Gou, et al., 2023). By contrast, freeze-dried samples showed similar intensities of sweet and fruity notes with raw samples, which was in line with Gou et al., (2023). There were two group in the clustered heatmap (**Figure 7-1(b)**), one group is raw, steamed and freeze-dried samples, another group is baked and fried samples, indicating that there was a dramatic difference in aroma profiles between the two clusters, baking and frying had a greater influence on the aroma characteristics in the raw samples than steaming and freeze drying.



(a)

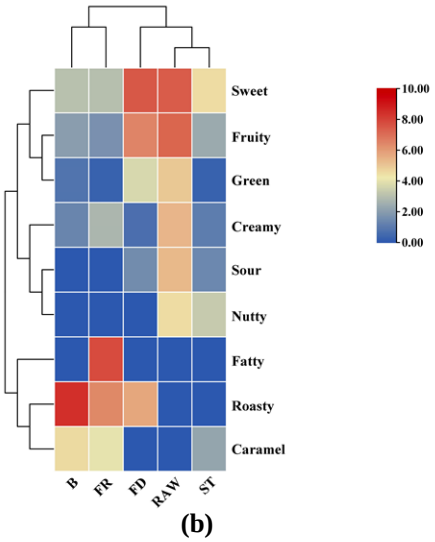


Figure 7-1 Sensory evaluation results based on quantitative descriptive analysis (QDA) (a) and the Hierarchical clustering heatmap plot (b).

3.2 Effect of different processing methods on the aroma compounds in red jujube

A total of 104 volatiles were identified in all samples through GC-MS/MS, including alcohols (6), aldehydes (20), ketones (10), acids (11), esters (24), lactones (10), pyrazines (9), alkenes (3) and others (11) (**Table 7-1**). The number of aroma compounds, in the steamed and fried samples was reduced from 55 compounds in raw red jujube to 34 and 53 compounds, respectively, and that in the baked and freeze-dried samples was increased to 56 compounds. Venn analysis showed that 17 compounds were detected in all samples. In addition, 13, 6, 9 and 2 compounds were identified only in fried, baked, freeze-dried and steamed samples, respectively (**Figure 7-2)**. These 47 specific compounds are presented in **Figure 7-3** through a network diagram.

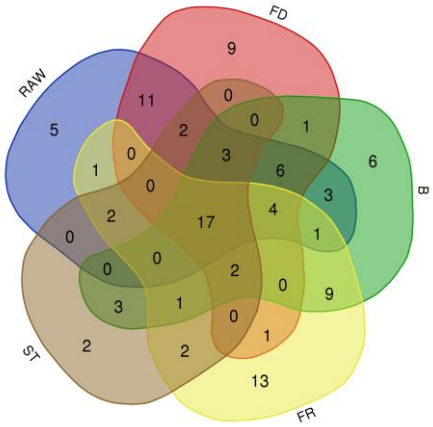


Figure 7-2 The venn diagram of aroma compounds identified in raw and different treated red jujube by GC-MS/MS.

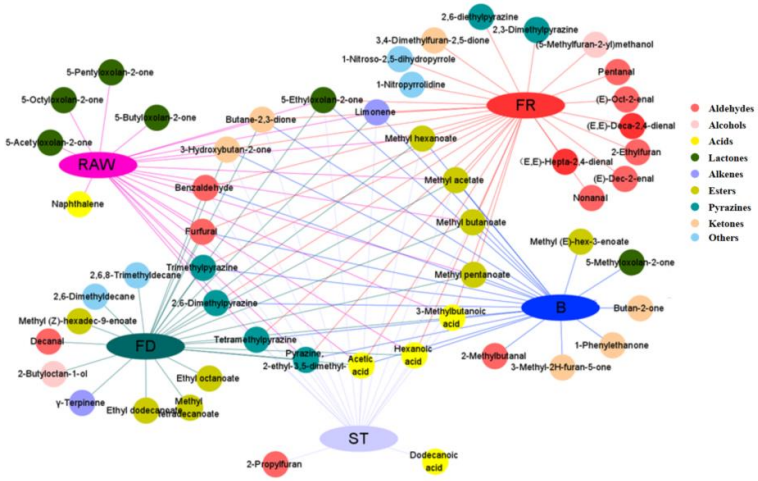


Figure 7-3 Aroma changes in red jujube after different treatments. Joint and special volatile compounds network diagram in all red jujube samples.

In the raw sample, esters had the highest content (15,332 μg/kg), followed by acids (8,924 μg/kg) and ketones (5,036 μg/kg) (**Table 7-1**). From the GC-O results, esters contributed fruity and sweet notes, acids contributed sour notes and ketones contributed creamy and buttery characteristics (**Table 7-2**), these results are consistent with Gou et al., (2021). Among these esters, methyl decanoate with middle fruity and green notes, ethyl decanoate with middle fruity notes and

methyl dodecanoate with strong sweet and fruity notes were key aroma-active compounds of red jujube (Gou et al., 2022). These compounds dominated the fruity, sweety and creamy notes of raw sample. Besides, 5-butyloxolan-2-one, 5-acetyloxolan-2-one, 5-pentyloxolan-2-one and 5-octyloxolan-2-one were detected only in raw jujube, and contributed a weak sweet note (**Figure 7-3**).

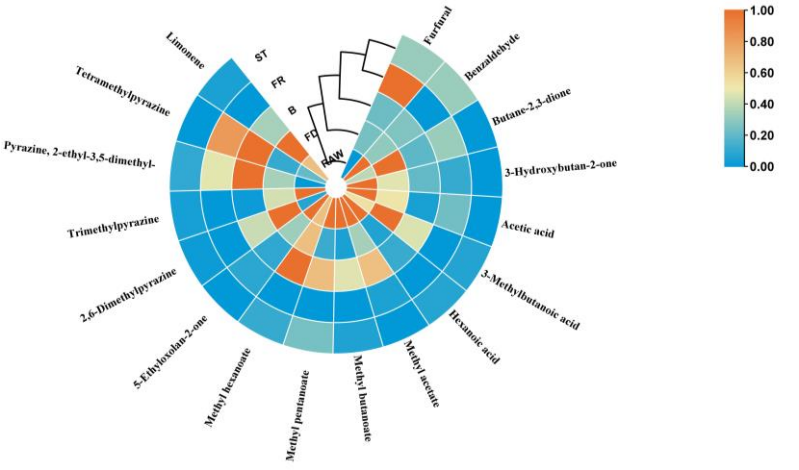


Figure 7-4 Aroma changes in red jujube after different treatments. The heatmap of joint volatile compounds in all red jujube samples.

Table 7-1 Volatile compounds identified in red jujube samples from different treatments.

Code	LRI([a]/[b])	Compounds	Identification	Concentration (μg/kg)				
				RAW	FD	B	FR	ST
		Alcohols						0
A1	1543/1553	Butane-2,3-diol	MS, RI, Std	97.33±13.83ab	113±1a	79.15±4.55b	52.42±2.79c	n.d
A2	1665/1669	Furan-2-ylmethanol	MS, RI	n.d	n.d	97.54±5.83b	229±2a	n.d
A3	1679/1684	Furan-3-ylmethanol	MS, RI	n.d	n.d	22.39±3.09b	149±8a	n.d
A4	1729/1720	(5-Methylfuran-2-yl)methanol	MS, RI	n.d	n.d	n.d	22.79±0.74	n.d
A5	1450/1448	Oct-1-en-3-ol	MS, RI, O, Std	96.57±4.84a	28.37±3.98b	n.d	n.d	n.d
A6	1855/1853	2-Butyloctan-1-ol	MS, RI	n.d	36.1±6.2	n.d	n.d	n.d
		Total		**194**	**178**	**199**	**453**	**0**
		Aldehydes						
B1	832/812	2-Methylpropanal	MS, RI	n.d	n.d	122±12b	167±2a	n.d
B2	890/880	2-Methylbutanal	MS, RI	n.d	n.d	54.92±1.4	n.d	n.d
B3	900/932	3-Methylbutanal	MS, RI, O, Std	n.d	99.21±11.61b	235±16a	213±1a	114±8b
B4	960/945	2-Ethylfuran	MS, RI, O	n.d	n.d	n.d	7.16±0.47	n.d
B5	980/974	Pentanal	MS, RI	n.d	n.d	n.d	38.96±0.89	n.d
B6	1014/1043	2-Propylfuran	MS, RI, Std	n.d	n.d	n.d	n.d	2.59±0.09
B7	1083/1078	Hexanal	MS, RI, O, Std	106±13a	n.d	n.d	89.37±1.98b	81.64±2.11b
B8	1216/1228	(*E*)-Hex-2-enal	MS, RI, Std	39.61±3.36a	n.d	n.d	32.82±0.3b	17.79±1.56c
B9	1230/1249	2-Pentylfuran	MS, RI, Std	n.d	n.d	n.d	94.63±3.01a	12.72±0.78b
B10	1283/1789	(*E*,*E*)-Deca-2,4-dienal	MS, RI, O	n.d	n.d	n.d	123±2	n.d
B11	1291/1287	Octanal	MS, RI, Std	40.09±1.26a	n.d	21.58±0.34c	32.35±1.2b	n.d
B12	1325/1318	(*E*)-Hept-2-enal	MS, RI,Std	16.93±3.32b	n.d	n.d	202±5a	n.d
B13	1392/1409	Nonanal	MS, RI, O, Std	n.d	n.d	n.d	63.07±0.82	n.d
B14	1429/1410	(*E*)-Oct-2-enal	MS, RI, Std	n.d	n.d	n.d	31.58±2.14	n.d
B15	1463/1479	Furfural	MS, RI, O, Std	89.09±8.82c	329±4b	308±1b	1054±34a	397±14b
B16	1497/1451	(*E*,*E*)-Hepta-2,4-dienal	MS, RI, O	n.d	n.d	n.d	139±5	n.d
B17	1519/1508	Benzaldehyde	MS, RI, O, Std	487±18a	197±12b	182±1b	69.69±3.76c	205±29b
B18	1520/1498	Decanal	MS, RI, Std	n.d	21.63±0.67	n.d	n.d	n.d

B19	1540/1615	(*E*)-Dec-2-enal	MS, RI	n.d	n.d	n.d	65.19±3.72	n.d
B20	1597/1582	5-Methyl furfural	MS, RI, O	n.d	n.d	39.8±0.11b	62.07±1.26a	n.d
		Total		**779**	**646**	**963**	**2485**	**831**
		Ketones						
C1	900/	Butan-2-one	MS, RI	n.d	n.d	30.18±2.95	n.d	n.d
C2	979/979	Butane-2,3-dione	MS, RI, O, Std	187±1b	320±12a	144±3c	172±4b	101±7d
C3	1061/1062	Pentane-2,3-dione	MS, RI,Std	n.d	n.d	46.53±0.41a	25.48±0.68b	n.d
C4	1286/1286	3-Hydroxybutan-2-one	MS, RI, O, Std	4698±72a	2910±218 b	2003±23c	1680±18cd	1294±128d
C5	1339/1342	6-Methylhept-5-en-2-one	MS, RI, Std	102±10a	74.08±11. 24b	n.d	n.d	n.d
C6	1378/-	3-Oxobutan-2-yl acetate	MS, RI, O, Std	49.07±4.54b	74.32±2.6 7a	n.d	n.d	n.d
C7	1547/1535	Cyclopent-4-ene-1,3-dione	MS, RI	n.d	n.d	8.64±0.2b	30.3±0.46a	n.d
C8	1670/1645	1-Phenylethanone	MS, RI	n.d	n.d	4.24±1.23	n.d	n.d
C9	1780/-	3,4-Dimethylfuran-2,5-dione	MS, RI	n.d	n.d	n.d	3.05±0.11	n.d
C10	1912/-	3-Methyl-2H-furan-5-one	MS, RI, O	n.d	n.d	18.33±1.37	n.d	n.d
		Total		**5036**	**3379**	**2255**	**1910**	**1395**
		Acids						
D1	1447/1429	Acetic acid	MS, RI, O, Std	4217±135a	2525±225 ab	878±15c	1522±26b	695±144c
D2	1528/1508	Propionic acid	MS, RI, Std	82.62±3.47a	113±12a	110±25a	126±2a	n.d
D3	1623/1628	Butanoic acid	MS, RI, Std	121±11.2a	102±19a	50.59±1.15b	n.d	32.78±1.63b
D4	1665/1680	3-Methylbutanoic acid	MS, RI, O, Std	217±0b	321±8a	199±5b	97.22±2.81c	113±14c
D5	1732/1762	Pentanoic acid	MS, RI, Std	121±11.2a	108±11a	n.d	n.d	70.71±12.37 b
D6	1753/1750	(*E*)-But-2-enoic acid	MS, RI, O, Std	44.17±2.90a	37.25±3.1 6b	n.d	n.d	13.82±2.84b
D7	1841/1849	Hexanoic acid	MS, RI, O, Std	3326±219a	458±26b	605±32b	255±68c	491±7b
D8	1943/1943	Heptanoic acid	MS, RI, Std	227±1a	n.d	224±12a	n.d	n.d
D9	2178/2171	Nonanoic acid	MS, RI, Std	111±12a	n.d	8.15±0.85b	n.d	n.d
D10	2320/2279	Decanoic acid	MS, RI, Std	458±10a	221±3b	142±4c	n.d	137±29c
D11	2487/2502	Dodecanoic acid	MS, RI, O, Std	n.d	n.d	n.d	n.d	139±182
		Total		**8924**	**3884**	**2218**	**2000**	**1693**
		Esters						

E1	828/810	Methyl acetate	MS, RI, Std	1155±113a	414±14c	777±20b	89.00±4.23d	23.67±1.36d
E2	902/899	Methyl propionate	MS, RI	888±19a	67.1±8.33 c	202±3b	n.d	n.d
E3	945/989	Methyl butanoate	MS, RI	1737±41a	169±16cd	857±6b	80.3±4.9d	186±13c
E4	1011/1018	Methyl 3-methylbutanoate	MS, RI	557±4a	25.58±1.0 9c	230±8b	n.d	n.d
E5	1078/1082	Methyl pentanoate	MS, RI	1406±103a	236±21cd	978±25b	72.11±7.14d	403±37c
E6	1181/1177	Methyl hexanoate	MS, RI, Std	3201±300b	3217±212 b	4754±325a	78.8±8.86d	593±55c
E7	1233/1241	Ethyl hexanoate	MS, RI, Std	174±12b	221±8a	n.d	n.d	n.d
E8	1245/1259	Methyl (*E*)-hex-3-enoate	MS, RI	n.d	n.d	26.04±2.2	n.d	n.d
E9	1272/1265	Hexyl acetate	MS, RI, O, Std	324±29.30a	162±12.5 8b	n.d	n.d	n.d
E10	1326/1342	Ethyl heptanoate	MS, RI, O, Std	113±9.90b	271±28.7 5a	n.d	n.d	n.d
E11	1372/1374	Methyl octanoate	MS, RI, Std	748±5b	1009±82a	64.1±0.29c	n.d	n.d
E12	1457/1441	Ethyl octanoate	MS, RI, Std	n.d	842±49	n.d	n.d	n.d
E13	1518/1536	Methyl nonanoate	MS, RI, Std	100±2b	149±1a	n.d	n.d	n.d
E14	1593/1636	Methyl decanoate	MS, RI, O, Std	1370±135b	5693±500 a	187±5c	n.d	n.d
E15	1623/1631	Methyl benzoate	MS, RI, Std	853±13a	60.96±6.4 8c	98.21±2.18b	n.d	n.d
E16	1638/1633	Ethyl decanoate	MS, RI, O, Std	788±5a	1095±2a	n.d	n.d	n.d
E17	1804/1834	Methyl dodecanoate	MS, RI, O, Std	1305±124b	4399±153 a	154±4c	n.d	1148±80b
E18	1815/1830	Methyl cyclopentenolone	MS, RI	n.d	n.d	n.d	75.99±8.05a	62.46±6.49a
E19	1833/1854	Methyl 3-phenylpropanoate	MS, RI	n.d	n.d	105±2a	n.d	6.04±0.82b
E20	1841/1849	Ethyl dodecanoate	MS, RI, O, Std	n.d	2940±4	n.d	n.d	n.d
E21	1959/-	Methyl (*Z*)-tetradec-9-enoate	MS, RI, O	575±4b	889±61a	n.d	n.d	n.d
E22	2016/2037	Methyl tetradecanoate	MS, RI, O, Std	n.d	380±25	n.d	n.d	n.d
E23	2201/2243	Methyl hexadecanoate	MS, RI, Std	38.25±3.39a	43.13±4.1 8a	n.d	n.d	n.d
E24	2264/2277	Methyl (*Z*)-hexadec-9-enoate	MS, RI, O	n.d	86.12±6.0 3	n.d	n.d	n.d
		Total		**15332**	**22369**	**8433**	**396**	**2423**

		Lactones						
F1	1108/-	4-Methyloxolan-2-one	MS, RI	28.15±0.24a	n.d	2.97±0.29b	n.d	n.d
F2	1590/1600	5-Methyloxolan-2-one	MS, RI	n.d	n.d	9.65±0.61	n.d	n.d
F3	1639/1602	Oxolan-2-one	MS, RI, Std	n.d	52.74±6.15b	33.64±1.65c	57.2±1.63ab	69.24±2.2a
F4	1690/1736	5-Ethyloxolan-2-one	MS, RI, O, Std	237±3a	123±4b	83.03±0.99c	81.26±5.55c	66.16±4.65c
F5	1810/1796	5-Propyloxolan-2-one	MS, RI, O, Std	30.75±2.89a	19.99±1.03b	7.46±2.63c	n.d	n.d
F6	1912/1936	5-Butyloxolan-2-one	MS, RI, O, Std	55.84±5.83	n.d	n.d	n.d	n.d
F7	2068/2096	5-Acetyloxolan-2-one	MS, RI, O	7.7±0.77	n.d	n.d	n.d	n.d
F8	2083/2063	5-Pentyloxolan-2-one	MS, RI, O	52.92±9	n.d	n.d	n.d	n.d
F9	2221/2238	5-Heptyloxolan-2-one	MS, RI, Std	n.d	20.8±2.34a	15.45±0.72b	n.d	n.d
F10	2395/2384	5-Octyloxolan-2-one	MS, RI	31.85±0.32	n.d	n.d	n.d	n.d
		Total		**444**	**216**	**152**	**138**	**136**
		Pyrazines						
G1	1263/1274	2-Methylpyrazine	MS, RI	n.d	n.d	33.17±0.77b	53.23±0.58a	n.d
G2	1321/1319	2,6-Dimethylpyrazine	MS, RI, O	23.75±1.48c	102±2a	52.57±3.05b	17.12±1.81c	19.19±1.26c
G3	1325/1346	2,3-Dimethylpyrazine	MS, RI, O	n.d	n.d	n.d	14.29±0.5	n.d
G4	1369/1399	2-Ethyl-5-methylpyrazine	MS, RI, Std	n.d	n.d	32.11±3.97a	n.d	17.78±0.24b
G5	1378/1363	2-Ethyl-6-methylpyrazine	MS, RI, O, Std	n.d	n.d	64.1±0.2a	n.d	30.76±3.66b
G6	1398/1413	Trimethylpyrazine	MS, RI, O, Std	92.99±9.39a	58.63±5.37b	32.54±0.14c	31.15±0.61c	33.88±5.17c
G7	1440/1440	2,6-Diethylpyrazine	MS, RI, Std	n.d	n.d	n.d	5.21±0.54	n.d
G8	1452/1464	2-Ethyl-3,5-dimethyl-pyrazine	MS, RI, O, Std	32.27±2.43d	58.72±5.67c	108±1a	68.47±4.69b	39.80±5.10d
G9	1462/1457	Tetramethylpyrazine	MS, RI, O, Std	52.68±4.15b	44.97±0.78c	114±3a	100±1a	36.39±1.09c
		Total		**202**	**264**	**437**	**290**	**178**
		Alkenes						
H1	949/1189	Limonene	MS, RI,Std	274±2b	405±38a	146±6c	9.02±0.04d	30.62±3.31d
H2	1245/1238	γ-Terpinene	MS, RI, Std	n.d	45.94±1.18	n.d	n.d	n.d
H3	1255/1254	Styrene	MS, RI, O, Std	181±13a	99.73±7.85b	90.33±3.72b	17.89±1.2c	n.d

		Total		**455**	**550**	**237**	**26.90**	**30.62**
		Others						
I1	1014/-	2,6,8-Trimethyldecane	MS, RI	n.d	39.38±3.62	n.d	n.d	n.d
I2	1095/1261	*p*-Cymene	MS, RI, Std	39.42±3.15a	45.3±3.43a	n.d	n.d	n.d
I3	1102/-	2,6-Dimethyldecane	MS, RI, O	n.d	107±2	n.d	n.d	n.d
I4	1164/1139	1,4-Xylene	MS, RI, Std	49.34±0.46a	45.75±3.17a	39.98±1.14a	13.56±1.04b	n.d
I5	1280/1707	Naphthalene	MS, RI, Std	26.95±3.73	n.d	n.d	n.d	n.d
I6	1394/-	1-Nitroso-2,5-dihydropyrrole	MS, RI	n.d	n.d	n.d	31.15±0.61	n.d
I7	1512/-	1-(Furan-2-yl)ethanone	MS, RI, O	n.d	n.d	37.22±1.71a	33.78±0.47b	n.d
I8	1740/-	Pyrazine-2-carboxamide	MS, RI	n.d	42.38±0.1b	n.d	66.43±0.83a	n.d
I9	1780/-	1-(4H-Pyridin-1-yl)ethanone	MS, RI, O	n.d	n.d	83.9±3.43b	99.62±0.74a	n.d
I10	1989/-	1-Nitropyrrolidine	MS, RI	n.d	n.d	n.d	114±10	n.d
I11	2278/-	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one	MS, RI, O	n.d	n.d	186±8a	92.92±2.36b	7.79±2.52c
		Total		**116**	**280**	**347**	**452**	**7.79**
		Total		**31483**	**31766**	**15239**	**8151**	**6694**

FD: freeze drying; B: baking; FR: frying; ST: steaming.

n.d: not detected.

[a] Linear retention index on DB-Wax columns were determined by *n*-alkanes.

[b] Linear retention index on DB-WAX column from the literature. And "-" indicated the compound lack of reliable linear retention index value in literature.

Table 7-2 Odor active value (OAV) and odour description by GC-O of volatile compounds in red jujube from different treatments.

Compounds	**RAW**		**FD**		**B**		**FR**		**ST**	
	OAV	**GC-O**	**OAV**	**GC-O**	**OAV**	**GC-O**	**OAV**	**GC-O**	**OAV**	**GC-O**
Alcohols										
Butane-2,3-diol	<1	-	<1	-	<1	-	<1	-	n.d	-
Furan-2-ylmethanol	n.d	-	n.d	-	/	-	/	-	n.d	-
Furan-3-ylmethanol	n.d	-	n.d	-	/	-	/	-	n.d	-

(5-Methylfuran-2-yl)methanol	n.d	-	n.d	-	n.d	-	/	-	n.d	-
Oct-1-en-3-ol	64.38	mushroom; M	18.91	mushroom; M	n.d	-	n.d	-	n.d	-
2-Butyloctan-1-ol	n.d	-	/	-	n.d	-	n.d	-	n.d	-
Aldehydes										
2-Methylpropana	n.d	-	n.d	-	/	-	/	-	n.d	-
2-Methylbutanal	n.d	-	n.d	-	/	-	n.d	-	n.d	-
3-Methylbutanal	n.d	-	/	-	/	-	/	corn flakes; W	/	corn flakes; W
2-Ethylfuran	n.d	-	n.d	-	n.d	-	/	caramel; M	n.d	-
Pentanal	n.d	-	n.d	-	n.d	-	3.25	-	n.d	-
2-Propylfuran	n.d	-	n.d	-	n.d	-	n.d	-	/	-
Hexanal	21.28	green, grass; W	n.d	-	n.d	-	17.87	green, grass; W	16.33	green, grass; W
(*E*)-Hex-2-enal	<1	-	n.d	-	n.d	-	<1	-	<1	-
2-Pentylfuran	n.d	-	n.d	-	n.d	-	15.77	-	2.12	-
(*E*,*E*)-Deca-2,4-dienal	n.d	-	n.d	-	n.d	-	1752	fatty; S	n.d	-
Octanal	/	-	n.d	-	/	-	/	-	n.d	-
(*E*)-Hept-2-enal	1.3	-	n.d	-	n.d	-	15.55	-	n.d	-
Nonanal	n.d	-	n.d	-	n.d	-	57.34	fatty; M	n.d	-
(*E*)-Oct-2-enal	n.d	-	n.d	-	n.d	-	10.53	-	n.d	-
Furfural	<1	-	<1	-	<1	-	<1	roasty; W	<1	roasty; W
(*E*,*E*)-Hepta-2,4-dienal	n.d	-	n.d	-	n.d	-	46.27	fatty; M	n.d	-
Benzaldehyde	<1	almond, berry; W	<1	almond, berry; W	<1	almond, berry; W	<1	almond, berry; W	<1	almond, berry; W
Decanal	n.d	-	7.98	-	n.d	-	n.d	-	n.d	-
(*E*)-Dec-2-enal	n.d	-	n.d	-	n.d	-	/	-	n.d	-
5-Methyl furfural	n.d	-	n.d	-	/	-	/	malty, baked; W	n.d	-

Ketones										
Butan-2-one	n.d	-	n.d	-	/	-	n.d	-	n.d	-
Butane-2,3-dione	187	butter, creamy, fruity; S	320	butter, creamy, fruity; S	144	butter, creamy, fruity; S	172	butter, creamy, fruity; S	101	butter, creamy, fruity; S
Pentane-2,3-dione	n.d	-	n.d	-	/	-	/	-	n.d	-
3-Hydroxybutan-2-one	336	butter, creamy, sweet; S	208	butter, creamy, sweet; S	143	butter, creamy, sweet; M	120	butter, creamy, sweet; W	92.45	butter, creamy, sweet; W
6-Methylhept-5-en-2-one	1.5	-	1.09	-	n.d	-	n.d	-	n.d	-
3-Oxobutan-2-yl acetate	/	-	/	butter, creamy, sweet; M	n.d	-	n.d	-	n.d	-
Cyclopent-4-ene-1,3-dione	n.d	-	n.d	-	/	-	/	-	n.d	-
1-Phenylethanone	n.d	-	n.d	-	/	-	n.d	-	n.d	-
3,4-Dimethylfuran-2,5-dione	n.d	-	n.d	-	n.d	-	/	burnt; M	n.d	-
3-Methyl-2H-furan-5-one	n.d	-	n.d	-	/	sweet; M	n.d	-	n.d	-
Acids										
Acetic acid	767	sour; S	459	sour; S	160	sour; M	277	sour; M	126	sour; M
Propionic acid	<1	-	<1	-	<1	-	<1	-	n.d	-
Butanoic acid	<1	-	<1	-	<1	-	n.d	-	<1	-
3-Methylbutanoic acid	<1	sour, putrid fruit, rancid, sweat; S	<1	sour, putrid fruit, rancid, sweat; S	<1	sour, putrid fruit, rancid, sweat; S	<1	sour, putrid fruit, rancid, sweat; S	<1	sour, putrid fruit, rancid, sweat; S
Pentanoic acid	<1	-	<1	-	n.d	-	n.d	-	<1	-

(*E*)-But-2-enoic acid	/	sweet; S	/	sweet; M	n.d		n.d		/	-
Hexanoic acid	3.73	sour; M	<1	sour; M	<1	sour; M	<1	sour; M	<1	sour; M
Heptanoic acid	<1	-	n.d	-	<1	-	n.d	-	n.d	-
Nonanoic acid	<1	-	n.d	-	<1	-	n.d	-	n.d	-
Decanoic acid	<1	-	<1	-	<1	-	n.d	-	<1	-
Dodecanoic acid	n.d	-	n.d	-	n.d	-	n.d	-	34.81	fruity; M
Esters										
Methyl acetate	<1	-	<1	-	<1	-	<1	-	<1	-
Methyl propionate	/	-	/	-	/	-	n.d	-	n.d	-
Methyl butanoate	/	sweet, fruity, floral; S	/	sweet, fruity, floral; S	/	sweet, fruity, floral; W	/	sweet, fruity, floral; W	/	sweet, fruity, floral; W
Methyl 3-methylbutanoate	/	-	/	-	/	-	n.d	-	n.d	-
Methyl pentanoate	/	-	/	-	/	-	/	-	/	-
Methyl hexanoate	45.72	-	45.96	-	67.92	-	1.13	-	8.47	-
Ethyl hexanoate	34.76	-	44.25	-	n.d	-	n.d	-	n.d	-
Methyl (*E*)-hex-3-enoate	n.d	-	n.d	-	/	-	n.d	-	n.d	-
Hexyl acetate	<1	fruity; M	<1	fruity; M	n.d	-	n.d	-	n.d	-
Ethyl heptanoate	595	fruity, brandy; M	1426	fruity, brandy; S	n.d	-	n.d	-	n.d	-
Methyl octanoate	3.74	-	5.04	-	<1	-	n.d	-	n.d	-
Ethyl octanoate	n.d	-	43.64	-	n.d	-	n.d	-	n.d	-
Methyl nonanoate	/	-	/	-	n.d	-	n.d	-	n.d	-
Methyl decanoate	319	fruity, green; M	1324	fruity, green; S	43.43	fruity, green; W	n.d	-	n.d	-
Methyl benzoate	11.68	-	<1	-	1.35	-	n.d	-	n.d	-
Ethyl decanoate	158	fruity; M	219	fruity; M	n.d	-	n.d	-	n.d	-
Methyl dodecanoate	870	sweet,	2933	sweet,	102	sweet,	n.d	-	765	sweet, jujube;

		jujube; S		jujube; S		jujube; S				S
Methyl cyclopentenolone	n.d	-	n.d	-	n.d	-	/	-	/	-
Methyl 3-phenylpropanoate	n.d	-	n.d	-	/	-	n.d	-	/	-
Ethyl dodecanoate	n.d	-	<1	sweet, fruity; S	n.d	-	n.d	-	n.d	-
Methyl (*Z*)-tetradec-9-enoate	/	-	/	sweet, caramel; S	n.d	-	n.d	-	n.d	-
Methyl tetradecanoate	n.d	-	/	honey; M	n.d	-	n.d	-	n.d	-
Methyl hexadecanoate	<1	-	<1	-	n.d	-	n.d	-	n.d	-
Methyl (*Z*)-hexadec-9-enoate	n.d	-	/	sweet; W	n.d	-	n.d	-	n.d	-
Lactones										
4-Methyloxolan-2-one	/	-	n.d	-	/	-	n.d	-	n.d	-
5-Methyloxolan-2-one	n.d	-	n.d	-	/	-	n.d	-	n.d	-
Oxolan-2-one	n.d	-	<1	-	<1	-	<1	-	<1	-
5-Ethyloxolan-2-one	<1	coconut, sweet; M	<1	coconut, sweet; M	<1	-	<1	-	<1	-
5-Propyloxolan-2-one	<1	caramel, sweet; M	<1	caramel, sweet; M	<1	caramel, sweet; W	n.d	-	n.d	-
5-Butyloxolan-2-one	8.59	sweet; W	n.d	-	n.d	-	n.d	-	n.d	-
5-Acetyloxolan-2-one	/	sweet; W	n.d	-	n.d	-	n.d	-	n.d	-
5-Pentyloxolan-2-one	5.46	sweet; W	n.d	-	n.d	-	n.d	-	n.d	-
5-Heptyloxolan-2-one	n.d	-	9.9	-	7.36	-	n.d	-	n.d	-
5-Octyloxolan-2-one	/	-	n.d	-	n.d	-	n.d	-	n.d	-
Pyrazines										
2-Methylpyrazine	n.d	-	n.d	-	<1	-	<1	-	n.d	-

2,6-Dimethylpyrazine	<1	nutty; W	<1	burnt, cocoa, pyrazine; S	<1	burnt, cocoa, pyrazine; S	<1	nutty; W	<1	nutty; W
2,3-Dimethylpyrazine	n.d	-	n.d	-	n.d	-	/	caramel, nutty; M	n.d	-
2-Ethyl-5-methylpyrazine	n.d	-	n.d	-	<1	-	n.d	-	<1	-
2-Ethyl-6-methylpyrazine	n.d	-	n.d	-	1.6	nutty; M	n.d	-	<1	-
Trimethylpyrazine	<1	nutty; M	<1	nutty; M	<1	burnt; M	<1	burnt; M	<1	burnt; M
2,6-Diethylpyrazine	n.d	-	n.d	-	n.d	-	<1	-	n.d	-
2-Ethyl-3,5-dimethyl-pyrazine	807	nutty, roasty; S	1468	nutty, roasty; S	2706	nutty, roasty; S	1712	nutty, roasty; S	995	nutty, roasty; S
Tetramethylpyrazine	<1	-	<1	-	<1	coca, roasty; M	<1	coca, roasty; M	<1	-
Alkenes										
Limonene	1.37	-	2.01	-	<1	-	<1	-	<1	-
γ-Terpinene	n.d	-	<1	-	n.d	-	n.d	-	n.d	-
Styrene	2.79	-	1.53	-	1.39	-	<1	-	n.d	-
Others										
2,6,8-Trimethyldecane	n.d	-	/	-	n.d	-	n.d	-	n.d	-
p-Cymene	7.87	-	9.04	-	n.d	-	n.d	-	n.d	-
2,6-Dimethyldecane	n.d	-	/	floral; W	n.d	-	n.d	-	n.d	-
1,4-Xylene	/	-	/	-	/	-	/	-	n.d	-
Naphthalene	4.49	-	n.d	-	n.d	-	n.d	-	n.d	-
1-Nitroso-2,5-dihydropyrrole	n.d	-	n.d	-	n.d	-	/	-	n.d	-
1-(Furan-2-yl)ethanone	n.d	-	n.d	-	/	caramel, roasty; S	/	caramel, roasty; S	n.d	-

Pyrazine-2-carboxamide	n.d	-	/	-	n.d	-	/	-	n.d	-
1-(4H-Pyridin-1-yl)ethanone	n.d	-	n.d	-	/	caramel, sweet; M	/	caramel, sweet; M	n.d	-
1-Nitropyrrolidine	n.d	-	n.d	-	n.d	-	/	-	n.d	-
3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one	n.d	-	n.d	-	/	caramel, roasty; S	/	caramel, roasty; S	/	caramel, roasty; W

FD: freeze drying; B: baking; FR: frying; ST: steaming.

n.d: not detected; "-": not perceived by GC-O; "/": the threshold not found; "S, M, W": the intensity of GC-O, "S": strong, "M": middle, "W": weak.

The different processing methods had significant effects on the volatile content and resulted in different aroma profiles. Baking, steaming and frying, as thermal processing methods, contributed unique aroma to red jujube after processing. Though the total volatile content was decreased after baking (51.59%), frying (74.11%) and steaming (78.74%), there was no obvious difference in the number of compounds perceived by GC-O in comparison with raw red jujube, except with steaming (**Table 7-1 and Table 7-2**). Baking showed the greatest loss of acid content (75.02%), followed by lactones (63.29%), ketones (55.22%) and esters (43.38%), resulting in weaker or no perception of sour, sweet, creamy and fruity notes in comparison with raw red jujube. By contrast, the pyrazine content increased more than two-fold, dominating the roasty characteristic of the baked red jujube. The GC-O results showed that caramel and roasty were the most frequently occurring aroma descriptors of baked red jujube, which agrees with the sensory evaluation results (**Figure 7-1**). In addition, 3-methyl-2H-furan-5-one with middle sweet note was detected only in baked red jujube. According to studies on baking products, roasty and caramel notes in baked red jujube are generally a result of the Maillard reaction in baked red jujube (Schoenauer & Schieberle, 2019). Ketones in raw red jujube, mainly including butane-2,3-dione and 3-hydroxybutan-2-one, could be the precursors, and acetic acid would be consumed as an intermediate of the Maillard reaction, then eventually pyrazines and other heterocyclic compounds would be formed (Gou, et al., 2023). This also explains the reason for the changes in the content of ketones, acids and pyrazines after baking (Gou, et al., 2023).

Unlike baking, frying produced the greatest loss of esters (97.34%), followed by acids (77.48%) and ketones (62.07%), while aldehydes, alcohols and pyrazines increased by 219%, 134% and 43.56%, respectively, and contributed fatty, caramel and roasty notes (**Table 7-1**). This was because not only the Maillard reaction produced heterocyclic compounds, but lipid oxidation and fatty acid degradation also produced unsaturated fatty aldehydes and fatty alcohols during frying. From **Figure 7-3**, (*E, E*)-deca-2,4-dienal, (*E, E*)-hepta-2,4-dienal, nonanal, (*E*)-oct-2-enal and (*E*)-dec-2-enal were detected only in fried red jujube and were perceived as fatty notes. These aldehydes were also found in fried mountain pepper oil (Ni et al., 2021), fried beans (S. Bi et al., 2021), French fries (L. Xu et al., 2022), and fried yellow croaker (Wei et al., 2023), and identified as the key aroma compounds of frying. Besides, some heterocyclic compounds and sulphur-containing compounds, for example, 2-ethylfuran, 2,6-diethylpyrazine, 2,3-dimethylpyrazine, 1-nitropyrrolidine,

1-nitroso-2,5-dihydropyrrole and (5-methylfuran-2-yl) methanol, all products of the Maillard reaction, contributed to a roasty note. For steamed red jujube, the proportion of esters declined sharply (83.73%), but esters were still the most abundant aroma compounds. It is noteworthy that alcohols were no longer detected after steaming, which could be due to evaporation with water vapour or thermal catalysed synthesis (Wei et al., 2023). Otherwise, dodecanoic acid and 2-propylfuran were only detected in steamed samples **(Figure 7-3)**.

Only the aroma content of freeze-dried sample increased. Similar with baking, acids had the highest loss (56.68%) after freeze drying, followed by lactones (52.66%) and ketones (32.90%) (**Table 7-1**). In contrast to the other treatments, the esters increased by 50.18% and the number of volatile compounds perceived through GC-O was the largest after freeze drying. Most of these esters were characterized as fruity and sweet (Gou et al., 2021). This was attributed to non-enzymatic esterification of alcohols and organic acids, amino acid metabolism and fatty acid oxidation, and this result was consisted with our previous studies (Gou, et al., 2023; Gou, et al., 2023). There were four esters detected only in freeze-dried red jujube, namely ethyl octanoate, ethyl dodecanoate, methyl (*Z*)-tetradec-9-enoate and methyl (*Z*)-hexadec-9-enoate. Among these, ethyl dodecanoate, contributed the sweety and fruity notes, was key aroma-active compound of freeze-dried sample (Gou et al., 2023).

There were 17 joint aroma compounds in all samples, including esters (4), pyrazines (4), acids (2), aldehydes (2), ketones (2), lactones (1) and alkenes (1), of which 3-hydroxy-2-butanone, butane-2,3-dione, 3,5-EDMP, hexanoic acid, 5-ethyloxolan-2-one, and 3-methyl-butanoic acid were key aroma compounds in raw sample (Gou et al., 2022). While, due to the change in contents of these key aroma compounds, the aroma profiles changed a lot after four different treatments. A decreased content of 3-hydroxy-2-butanone, butane-2,3-dione and acetic acid and an increased content of furfural and 3,5-EDMP were attributed to Maillard reaction that occurred in the four thermal treatments (**Figure 7-4**).

3.3. Identify the aroma-active compounds in different samples through OAVs

The contribution of odour active volatiles to the overall aroma of the different samples analysed through a Sankey graph based on their OAVs (**Figure 7-5**). There were 23, 18, 11, 13 and 9 aroma-active compounds of raw, freeze-dried, baked, fried

and steamed red jujube, respectively (OAV ≥ 1) (**Table 7-2**). It was observed that methyl dodecanoate, perceived as sweet and fruity notes by GC-O and were key aroma-active compound in raw and freeze-dried sample in our previous study (Gou et al., 2022; Gou et al., 2023), had the highest OAV (870) in raw red jujube. Moreover, butane-2,3-dione, 3-hydroxybutan-2-one, acetic acid, ethyl heptanoate, methyl decanoate, ethyl decanoate and 3,5-EDMP had higher OAVs and contributions to the aroma in raw samples. Although esters, including methyl dodecanoate (OAV = 2933), ethyl heptanoate (OAV = 1426) and methyl decanoate (OAV = 1324), were the main contributors to the aroma of freeze-dried samples, the OAV of 3,5-EDMP (1468) was markedly increased after freeze drying, which resulted the in changes in the aroma profile, whereas (*E*, *E*)-2, 4-deca-dienal (OAV = 1752) and 3,5-EDMP (OAV = 1712) were the dominant contributors to the aroma of fried red jujube.

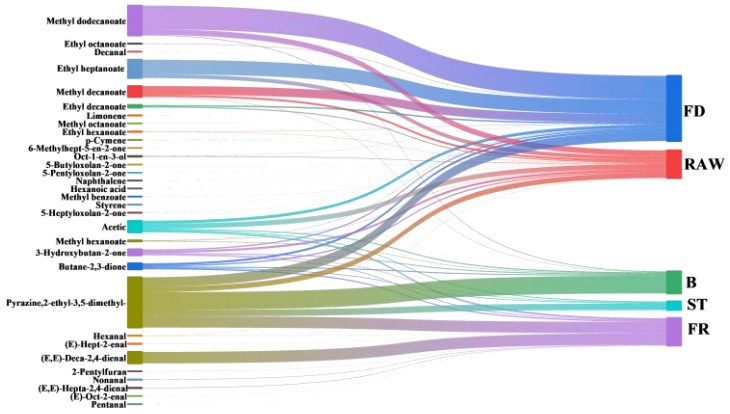


Figure 7-5 Sankey diagram based on OAV values of all red jujube samples.

On the other hand, 3,5-EDMP had the highest OAV (2706 and 995, respectively) in the baked and steamed red jujube, while it dominated the roasty note in the baked sample and the nutty note in the steamed sample (**Table 7-2)**. It was noted that 3,5-EDMP had the aroma contribution to all five samples, but the aroma characteristic was unique to each sample. A possible reason was that variations in the content of different compounds could result in different aroma characteristics, and the interaction between aroma compounds could affect the perception of the compounds in different samples.

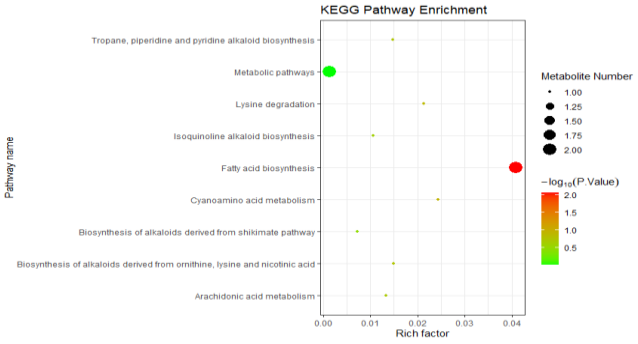
3.4. Untargeted metabolome by HPLC-MS/MS

The HPLC-MS/MS was further applied to illustrate the difference in non-volatile metabolites of red jujube. A total of 157 significant differential metabolites (VIP > 1, $p < 0.05$, absolute Log_2FC (fold change) > 3 or < 0.33, 92 up-regulated, 65 down-regulated) were identified in positive-ion and negative-ion modes in all samples, included amino acids and their derivatives (13), benzenoids (11), organoheterocyclic compounds (23), organic acids and derivatives (8), organic oxygen compounds (15), lipids and lipid-like molecules (50), alkaloids and their derivatives (3), nucleosides, nucleotides and their analogues (6), phenylpropanoids and polyketides (11) and others (17) (**Table 7-3**). From **Table 7-3**, organic oxygen compounds, as well as lipids and lipid-like molecules, were the superclass with the most up- and down-regulated metabolites in all processed samples, indicating carbohydrates, some carbonyl compounds, as well as lipids were the major precursors of metabolites and mainly involved in lipid oxidation and Maillard reactions.

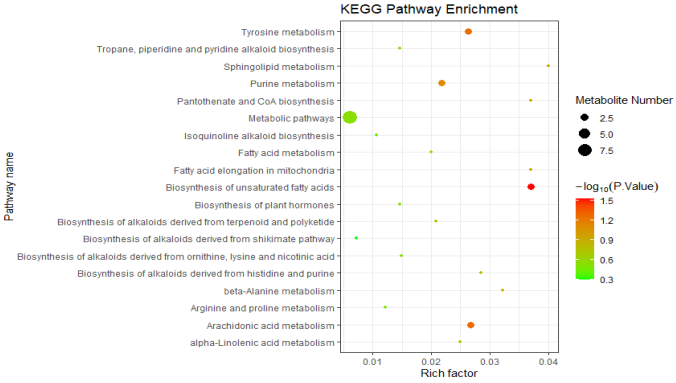
Table 7-3 The classes and numbers of important differential metabolites of red jujube detected in different treatments (Up: up- regulated; Down: down-regulated).

Superclass	FD-RAW		B-RAW		FR-RAW		ST-RAW		Up	Down	Total
	Up	Down	Up	Down	Up	Down	Up	Down			
Alkaloids and derivatives	1	0	1	0	1	0	0	0	**3**	**0**	**3**
Amino acids and derivatives	2	1	5	0	4	0	1	0	**12**	**1**	**13**
Benzenoids	2	2	3	1	3	0	0	0	**8**	**3**	**11**
Lipids and lipid-like molecules	4	8	7	4	9	9	3	6	**23**	**27**	**50**
Nucleosides, nucleotides and analogues	2	0	2	0	2	0	0	0	**6**	**0**	**6**
Organic acids and derivatives	0	0	3	0	4	1	0	0	**7**	**1**	**8**
Organic oxygen compounds	1	1	1	3	2	3	0	4	**4**	**11**	**15**
Organoheterocyclic compounds	0	1	6	2	8	3	0	3	**14**	**9**	**23**
Phenylpropanoids and polyketides	2	1	1	1	1	2	0	3	**4**	**7**	**11**
Others	1	0	2	2	5	2	3	2	**11**	**6**	**17**
Total	**15**	**14**	**31**	**13**	**39**	**20**	**7**	**18**	**92**	**65**	**157**

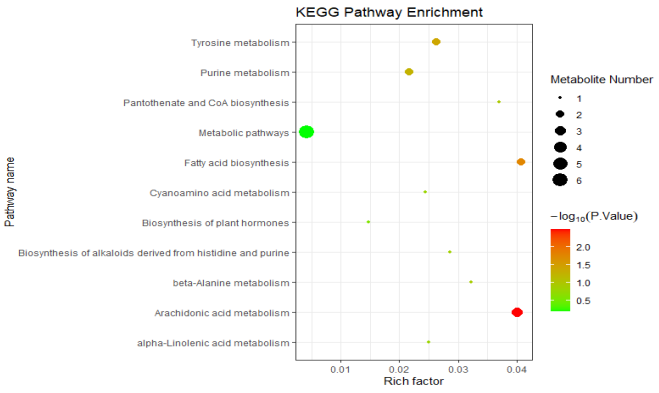
KEGG functional and pathway enrichment of differential metabolites were performed to illustrate the difference in differential metabolites in red jujube among different treatments. The results illustrated that 21 differential metabolites annotated in KEGG in freeze-dried samples - arachidonic acid metabolism, fatty acid biosynthesis and tyrosine metabolism were obviously enriched ($p < 0.05$, **Figure 7-6 (a)**). In baked red jujube, the metabolism of fatty acid, α-linolenic acid, tyrosine, arachidonic acid, purine and isoquinoline alkaloid biosynthesis were obviously enhanced ($p < 0.05$, **Figure 7-6(b)**), whereas in the fried and steamed samples, only biosynthesis of unsaturated fatty acids and fatty acid biosynthesis, respectively, were significantly enriched ($p < 0.05$, **Figure 7-6(c)(d)**). In general, fatty acids had a significant influence on aroma formation in red jujube during different treatments.



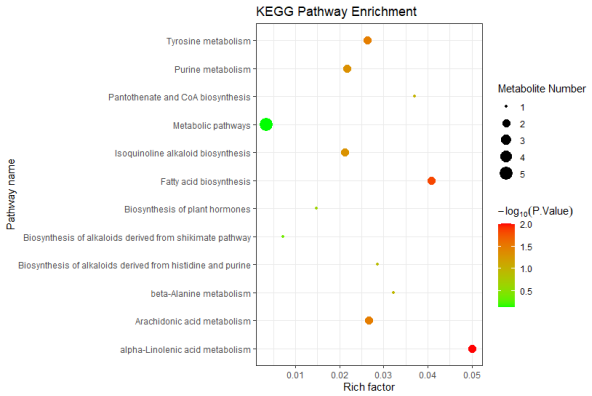
(a) Freeze drying



(b) Baking



(c) Frying



(d) Steaming

Figure 7-6 KEGG enrichment in red jujube after different treatments: (a) freeze drying vs. raw, (b) baking vs. raw, (c) frying vs. raw and (d) steaming vs. raw.

3.5. Potential transformation pathways of key odour-active compounds after different treatments

By comparison with the KEGG database, differential metabolites were identified and metabolic pathways in each treatment were significantly enriched, the potential formation pathways of key aromas in red jujube after different treatments were predicted (**Figure 7-7**). As a result of all treatments, fatty acids were significant precursors of esters and aldehydes. In addition, aroma compounds, such as some pyrazines, furans and DDMP, which might be involved in the Maillard reaction,

were presented in the processed samples but not in the raw ones. Therefore, this study focused on the pathway of lipid oxidation and Maillard reaction.

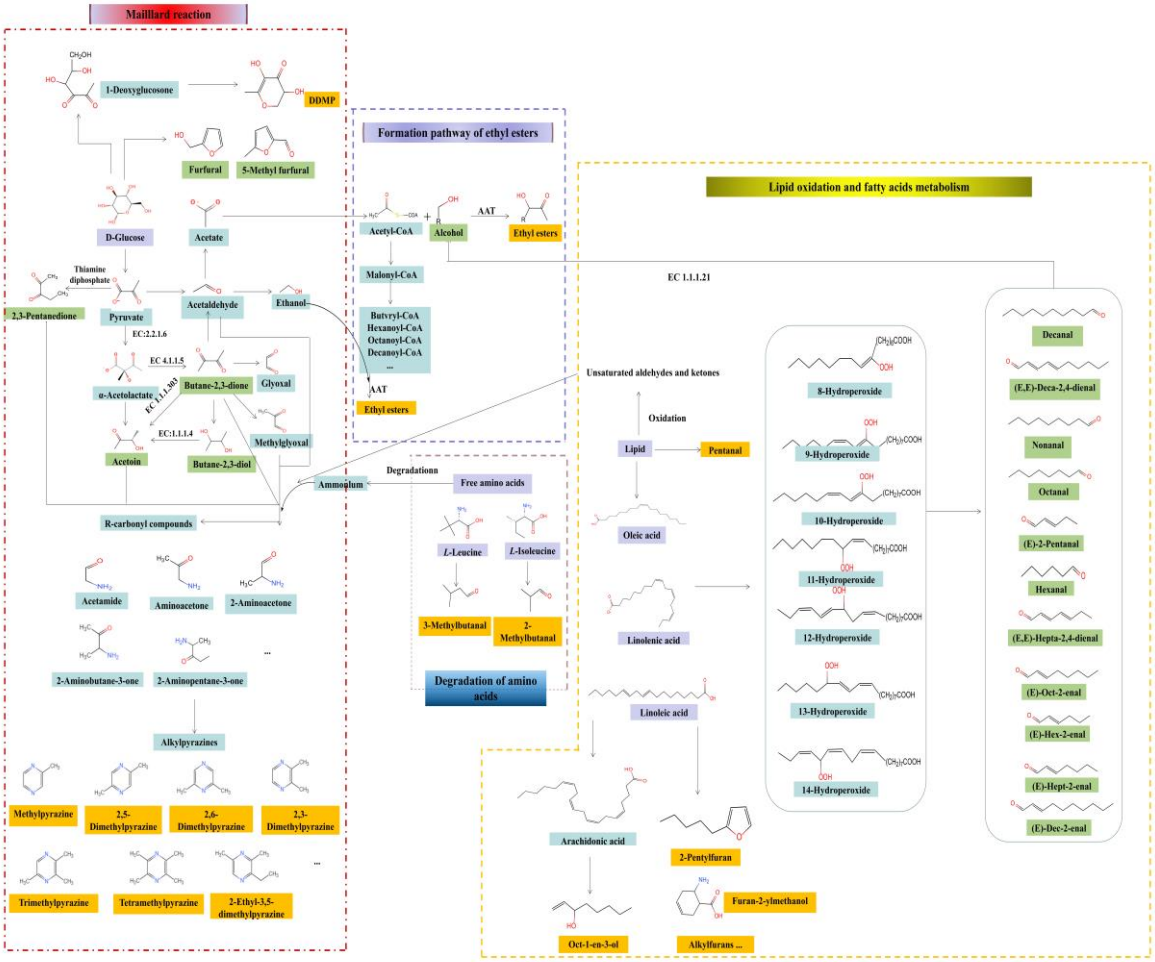


Figure 7-7 The sketch map of potential source of the aroma active compounds in different processed red jujube based on KEGG and reference. The purple, yellow and blue represented substrates, final aroma compounds and intermediate, respectively. The green represented not only the intermediate but also the substrates or final products.

According to the above-mentioned aroma compound analysis, aldehydes were important compounds in the fried sample, especially nonanal, (*E*,*E*)-deca-2,4-dienal and (*E*,*E*)-hepta-2,4-dienal which are mainly generated from the oxidation of unsaturated fatty acids (Hu et al., 2022; Wei et al., 2023). Generally, mono and poly

unsaturated fatty acids, such as oleic, linoleic, linolenic and arachidonic acids, can undergo auto/enzyme oxidation to form various hydroperoxides (*n*-ROOH), and then the carbon-carbon bonds of the hydroperoxides were broken by alkoxy radical α/β-scission, resulting in the formation of volatile, such as aldehydes (Du et al., 2020; Hidalgo & Zamora, 2019). Octanal, nonanal and decanal were generated from the homolysis of 8-ROOH, 10-ROOH and 11-ROOH, respectively (**Figure 7-7**). Hexanal was the oxidation product of linoleic acid and arachidonic acids, while linolenic could form more isomers, such as 9- and 14- hydroperoxides, leading to (*E*,*E*)-hepta-2,4-dienal, then (*E*)-oct-2-enal would be formed via retro-aldolization of (*E*,*E*)-deca-2,4-dienal, other 2-alkenal, such as (*E*)-hept-2-enal, (*E*)-dec-2-enal and (*E*)-hex-2-enal were also derived from cleavage via *α/β*-homolysis (Du et al., 2020; Hu et al., 2022; Sohail et al., 2022). In addition, alkyl furans, such as 2-pentylfuran, 2-ethylfuran and 2-propylfuran, are mainly generated from linoleic acid (Frank et al., 2020). Moreover, the aliphatic aldehydes through rearranged and cyclized can produce the furans, similarly, sugar also can produce furans through thermal degradation (Hu et al., 2022). In contrast to the above-mentioned aldehydes, 3-methylbutanal can be derived from leucine degradation and 2-methylbutanal can be derived from isoleucine degradation (Zhao et al., 2022). Furthermore, some aldehydes and ketones, which are generated from oxidation of fatty acids, could engaged in the Maillard reaction. Otherwise, the produced aldehydes could undergo further conversion to the corresponding alcohols, and these alcohols could form esters (**Figure 7-7**).

Esters also had a significant contribution to aroma profiles in raw, freeze-dried and steamed samples. The content of esters, especially that of ethyl esters, included ethyl heptanoate, ethyl decanoate, ethyl dodecanoate, ethyl hexanoate and ethyl octanoate, which contributed fruity and sweety note to freeze-dried sample, and increased after freeze drying. Generally, ester synthesis can be divided into three pathways. The first pathway uses alcohols and acids as substrates and performs the esterification reaction catalysed via ester synthase, the second one uses alcohols and aldehydes perform hemiacetal dehydrogenation, and the third one is the synthesis of ethyl esters catalysed by alcohol acyltransferases (AAT) using alcohol and acyl-CoA as substrates (Shi et al., 2021; Zhao et al., 2023). Alcohols are mainly derived from fatty acid oxidation, pyruvate metabolism and amino acid catabolism, while acyl-CoA could be generated from pyruvate metabolism (Ma et al., 2020; Shalit et al., 2001; Shi et al., 2021) (**Figure 7-7**). Additionally, acyl-CoA could be generated from

fatty acid oxidation and amino acid metabolism (Schwab et al., 2008b).

Pyrazines are key aroma compounds of baked red jujube, and it is worth mentioning that 3,5-EDMP made a significant contribution to the aroma in all five samples. In addition, 1-(furan-2-yl) ethenone, 1-(4H-pyridin-1-yl) ethenone and DDMP (3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one) with caramel notes were perceived and detected in baked and fried red jujube, and DDMP was also presented in the steamed samples. These heterocyclic compounds are all derived from the Maillard reaction, and α-aminocarbonyl compounds can be formed through the reaction between α-dicarbonyl compounds and amino acids. Following the dihydropyrazines can be derived through the condensation of two α-aminocarbonyl compounds (**Figure 7-7**). Finally, the alkylpyrazines can be formed through the oxidation of the dihydropyrazines or reaction between deprotonated dihydropyrazines and carbonyl compounds (Hu et al., 2022; Ma et al., 2022; Zhang et al., 2020). Meanwhile, raw red jujube had an abundant content of butane-2,3-diol, butane-2,3-dione and 3-hydroxybutan-2-one, that served not only as the substrates but also as intermediate products of the Maillard reaction (**Figure 7-7**). Furthermore, glucose, sucrose and fructose, the three main sugars in red jujube (Gou, et al., 2023), which provide rich substrate source for the Maillard reaction. As a result of the thermal degradation of sugars, 5-methyl furfural can be generated (Delatour et al., 2020). Finally, the precursor of DDMP, namely 1-deoxyglucosone (1-DG), was also generated from glucose (H. Li et al., 2019) (**Figure 7-7**). In conclusion, red jujube involves the Maillard reaction, amino acid degradation, lipid oxidation, fatty acid metabolism and their complex interaction, in which different aromas are formed depending on the processing methods.

4.Conclusion

The aroma in red jujube as affected by freeze drying, baking, frying and steaming was investigated in this study. The aroma compound content of red jujube decreased after baking, frying and steaming, but increased after freeze drying. Aldehydes increased obviously and dominated a fatty aroma characteristic of fried red jujube, pyrazines content increased and contributed the roasty notes of baked red jujube. In addition, the creamy and sour notes decreased after all processing treatments. Ester content increased significantly and contributed to the fruity and sweet notes. 3,5-EDMP also played a key role in freeze-dried sample. These were the results of the

interaction of fatty acid metabolism and the Maillard reaction. Lastly, freeze-dried samples had a similar aroma profile to raw red jujubes, which could have a positive effect on overall aroma acceptance.

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Chapter VIII. General discussion, conclusion and perspective

1. General discussion

1.1 Analysis of volatile compounds

Red jujube has a pleasant aroma, which is one of the important factors to attract consumers and enhance market competitiveness. The main aroma compounds in red jujube are aldehydes, alcohols, acids, esters, ketone, lactones, pyrazines, olefins.

In the raw sample, esters had the highest content, contributed the fruity, sweet, and floral notes for red jujube. Methyl decanoate, ethyl decanoate and methyl dodecanoate were identified as the key aroma-active compounds in red jujube, meanwhile, ethyl heptanoate, ethyl dodecanoate and hexyl acetate also been key aroma-active compounds in red jujube after freeze drying. Among all the processing methods in this study, only the ester content in red jujube increased after freeze drying, and the content decreased after other processing methods (baking, steaming and frying).

Acids were the most numerous class of volatile compounds detected in the raw red jujube. The most abundant acids were acetic acid and hexanoic acid, with sour note. But they did not contribute a strong sour profile to red jujube due to the higher threshold. Otherwise, hexanoic acid, 3-methyl-butanoic acid and (*E*)-but-2-enoic acid were identified the key aroma-active compounds in red jujbe, they contributed the “sour and sweet” notes to red jujube (chapter 1). However, the contents of acids decreased significantly after freeze drying, baking, frying and steaming. In Song et al. (2020) study, red jujube had the highest content of total acids after constant lower temperature of freeze drying (25 ◦C). The different result might be due to the totally different drying condition. The processing methods used in this study could promote the chemical reaction occurred, and leading the acids transformed to esters. In addition, acids might be discharged by vacuum pump, due to they have lower vapor pressure and easily vaporized.

As the second most abundant class of volatile compound in raw red jujube, ketones also contributed the most OAVs to the overall aroma of raw red jujube. 3-hydroxy-2-butanone with butter note, butane-2,3-dione with creamy note, 6-methyl-5-hepten-2-one with sweety note and 3-oxobutan-2-yl acetate were key aroma-active compounds in raw red jujube. Similar to acids, most of ketones also decreased obviously after freeze drying, baking, frying and steaming. These ketones as α-dicarbonyl compounds could participate in the Maillard reaction and form pyrazines,

such as butane-2,3-dione and 3-hydroxybutan-2-one (Mei et al., 2007; Xiao et al., 2018).

Aldehydes have green, fatty, grassy, and fresh characteristics. The furfural was increased remarkably, because in the later stage of freeze drying, the red jujube temperature gradually close to the heating plate temperature (65 ℃), resulted the degradation of carbohydrates or Maillard reaction. In fried red jujue, (*E, E*)-deca-2,4-dienal, (*E, E*)-hepta-2,4-dienal, nonanal, (*E*)-oct-2-enal and (*E*)-dec-2-enal were detected perceived as fatty notes. These aldehydes were also found in fried mountain pepper oil (Ni et al., 2021), fried beans (S. Bi et al., 2021), French fries (L. Xu et al., 2022), and fried yellow croaker (Wei et al., 2023), and identified as the key aroma compounds of frying. They always generated from lipid oxidation and fatty acid degradation during frying.

Lactones could be generated as a result of hydroxyl acid intramolecular esterification (Al-Dalali et al., 2020) or formed from the reaction between glycine and D-glucose in slightly acidic (Keyhani and Yaylayan, 1996). 5-Propyloxolan-2-one, 5-butyloxolan-2-one and 5-ethyloxolan-2-one were identified as key aroma compounds in raw red jujube. Besides, 5-heptyloxolan-2-one appeared from stage 7 of freeze drying, was also identified as the key aroma compound of freeze-dried red jujube, which might be generated from the β-oxidation of fatty acids (Xi et al., 2012).

Pyrazines are important volatile compounds in raw and processed red jujube in this study. Pyrazines contributed to important aroma characteristics due to their low thresholds, especially 3,5-EDMP, which was the major contributor of nut notes in red jujube. Meanwhile, the increase of pyrazine content may also lead to the enhancement of roast note of freeze-dried, baked, steamed and fried red jujube. Pyrazines compounds are generally resulting from the Maillard reaction, which are more favorable at high temperature. In this study, the treatment methods applied all had higher temperatures, and red jujube is rich in aroma precursors, which provide conditions for the formation of pyrazine.

1.2 Potential formation pathways of odour active compounds

Red jujube contains rich aroma precursors, including amino acids, fatty acids and reducing sugars (J. Song et al., 2019), which could provide a variety of metabolic pathways and chemical reaction, such as Maillard reaction for the aroma formation of freeze-dried red jujube. Fatty acids are the precursors of most aliphatic alcohols,

aldehydes, ketones and esters that have a variety of oxidation pathways, among which lipoxygenase (LOX) oxidation pathway is involved in the synthesis of aroma compounds with green note (C-6 and C-9 aldehydes and alcohols) (Boukobza et al., 2001). Reducing sugar is also a precursor for the metabolic synthesis of alcohols, acids, esters. During anaerobic respiration, monosaccharides are converted to pyruvate, which is catalyzed by dehydrogenases to form acetyl-CoA and further ester compounds (El Hadi et al., 2013; Schwab et al., 2008a). In addition, amino acids could also form esters by acetyl-CoA or form pyrazines by Maillard reaction with reducing sugar (Gonda et al., 2010).

According to the above-mentioned volatile aroma compounds analysis, esters played an important role in aroma of raw, freeze-dried and steamed samples. Generally, ester synthesis can be divided into three pathways. The first pathway uses alcohols and acids as substrates and performs the esterification reaction catalysed via ester synthase, the second one uses alcohols and aldehydes perform hemiacetal dehydrogenation, and the third one is the synthesis of ethyl esters catalysed by alcohol acyltransferases (AAT) using alcohol and acyl-CoA as substrates (Shi et al., 2021; Zhao et al., 2023).

Pyrazines also had a significant contribution to raw, freeze-dried and baked red jujube. Especially, 3,5-EDMP identified as key aroma compound in raw and freeze-dried red jujube. Generally, the pyrazines compounds are all derived from Maillard reaction. Firstly, α-aminocarbonyl compounds can be formed through the reaction between α-dicarbonyl compounds and amino acids. Following the dihydropyrazines can be derived through the condensation of two α-aminocarbonyl compounds. Finally, the alkylpyrazines can be formed through the oxidation of the dihydropyrazines or reaction between deprotonated dihydropyrazines and carbonyl compounds (Hu et al., 2022; Ma et al., 2022; Zhang et al., 2020). Combined the results of chapter 5, 6 and 7, glucose, sucrose and fructose, the three main sugars in red jujube (Gou, et al., 2023), which provide rich substrate source for the Maillard reaction. Besides, raw red jujube had an abundant content of butane-2,3-diol, butane-2,3-dione and 3-hydroxybutan-2-one, that served not only as the substrates but also as intermediate products of the Maillard reaction. Butane-2,3-dione, pentane-2,3-dione, pyruvic acid, isopropyl alcohol, acetone and 2-hydroxypropionic acid had the significant correlation with 3,5-EDMP. In red jujube matrix, the dominant formation of 3,5-EDMP is condensation of 2-aminopentan-3-one produced

by pentane-2,3-dione and aminoacetone produced by the Strecker degradation of methylglyoxal to form dihydropyrazine, which is then oxidised to form 3,5-EDMP.

Aldehydes were aroma active compounds in fried red jujube, especially nonanal, (*E,E*)-deca-2,4-dienal and (*E,E*)-hepta-2,4-dienal which are mainly generated from the oxidation of unsaturated fatty acids (Hu et al., 2022; Wei et al., 2023). Generally, mono and poly unsaturated fatty acids, such as oleic, linoleic, linolenic and arachidonic acids, can undergo auto/enzyme oxidation to form various hydroperoxides (*n*-ROOH), and then the carbon-carbon bonds of the hydroperoxides were broken by alkoxy radical α/β-scission, resulting in the formation of volatile, such as aldehydes (Du et al., 2020; Hidalgo & Zamora, 2019). Octanal, nonanal and decanal were generated from the homolysis of 8-ROOH, 10-ROOH and 11-ROOH, respectively. Hexanal was the oxidation product of linoleic acid and arachidonic acids, while linolenic could form more isomers, such as 9- and 14- hydroperoxides, leading to (*E,E*)-hepta-2,4-dienal, then (*E*)-oct-2-enal would be formed via retro-aldolization of (*E,E*)-deca-2,4-dienal (Du et al., 2020; Hu et al., 2022; Sohail et al., 2022).

The formation of aroma is a complex process, not only the Maillard reaction, fatty acid oxidation reaction and enzyme reaction, but also the result of multiple reactions. For example, some volatile oxidation products of fatty acids, such as acids, ketones, alcohols, would also react with the intermediate products of Maillard reaction to generate flavor compounds and contribute to the overall aroma. They might generate some pyrazines and so on (Liu et al., 2020).

1.3 Analysis methods of volatile compounds

Aroma is an important quality of food, which is a complex mixture of volatile compounds. To date, numerous volatile compounds have been identified in food. However, because the content of volatile compounds is trace in food, not all components have a contribution to food. The concentration of most compounds needs to reach above its threshold to be perceived. Only these few compounds can contribute important effects to food. Volatile components with this property are called key aroma or aroma active component. Professor Peter Schieberle proposed the Molecular sensory science technology in 2007, which provides a better way to identify the key aroma compounds (Steinhaus & Schieberle, 2007). Untill now, the application of molecular sensory science has been reported in the identification of key aroma of baijiu, juice, mulberry fruit, tea, roasted peas and so on (S. Bi et al.,

2020; Fricke & Schieberle, 2020; Tan et al., 2019; Y. Xu et al., 2022; Wentao Zhang et al., 2019c; J. C. Zhu et al., 2018). Therefore, GC-O, GC-MS, OAV and DFA were firsty applied to screen aroma active compounds of red jujube before and after freeze-drying in this study. Then combined with reconstitution and omission test and QDA to identify the key aromas in raw and freeze-dried red jujube.

In order to understand the formation of the aroma of freeze-dried red jujube, correlation analysis between aroma compounds, aroma precursors and related enzyme activities were conducted. Correlation heatmap is a common graphical tool for visualizing correlations. In this study, correlation heatmap was used to analyse the correlation between enzyme activities and fatty acids. From the results, LOX activity showed a significantly positive correlation with palmitic acid, oleic acid, linoleic acid and α-linolenic acid; ADH showed a positive correlation with lauric acid; AAT showed a positive correlation with oleic acid. They may be related to the formation of aldehydes, alcohol and esters (Shuang Guo et al., 2022; Schwab et al., 2008b). Besides, the correlation heatmap was also employed to investigate the pyrazine formation process. The volatile compounds during the reaction were subjected to Pearson correlation analysis. The results showed butane-2,3-diol, hydroxyacetone and propylene glycol showed a highly significant correlation with pyrazines in both pH 5.5 and pH 7.8 models. Besides, butane-2,3-dione, pentane-2,3-dione, pyruvic acid, isopropyl alcohol, acetone and 2-hydroxypropionic acid had the significant correlation with 3,5-EDMP in pH 5.5 model. The results were in line with previous researches (Boekel, 2006; Jiang et al., 2022; A. N. Yu & Zhang, 2010; Huaizhi Zhang et al., 2020; Tong Zhou et al., 2022). That showed the correlation heatmap analysis was a useful and powerful analytical technique to analyse the correlation between aroma and precurors, and can provide ideas for the formation of aroma in red jujube.

The network correlation analysis is also a graphical tool for visualizing correlations that has been widely used in recent years. In this study, through the network correlation, Lys, Leu Arg, Ser, Val, Gly, Pro, Glu and Cys were identified as the main aroma precurors for aroma-active aroma compounds of freeze-dried red jujube. As compounds that contribute greatly to the aroma of freeze-dried jujube, pyrazine compounds were mainly positively correlated with Gly, Lys, Val, Leu, Arg and Glu. This is consistent with Yu et al., (2021) and Kocadağlı et al., (2021).

The Mantel test is another graphical tool for visualizing correlations. The Mantel

test between each of factors (reducing sugar, enzyme activies, FFA, FAA, and temperature) and different classes of aroma compounds were performed, aiming to determin which factors were significantly correlated with aroma compounds. Through the Mantel test, the amino acids and fatty acids could not only affect the volatile compounds alone, but also their interactions could affect the overall aroma to a great extent, which is mainly played by Maillard reaction. In Maillard reaction, amino compounds could be provided by amino acids, while carbonyl compounds could be converted from reducing sugar or fatty acids (L. Hou et al., 2017). In addition, temperature was also a key influencing factor in the aroma formation during freeze drying which is a complex process involved enzyme reaction and non-enzyme reaction. As reported in W. Li et al., (2022), temperature was also an important parameter in aroma formation during drying processing of shiitake mushrooms, and they inferred the aroma formation was dominated by enzymatic reactions in the pre-drying period and by non-enzymatic reactions in the post-drying period, which were all driven by temperature. In our study, combined the correlation value, the influence degree of factors on the aroma formation of freeze-dried jujube was ranked as temperature > enzyme activity > fatty acids > amino acids.

After screening out the key aroma of freeze-dried red jujube through molecular sensory science and dominated precursors through correlation analysis, a solid-state model system was established to study the formation of pyrazine. In previous studies, much of them on the formation of pyrazines were based on liquid model system and under the reaction temperature above 100 ^{o}C (S. Deng et al., 2022; Guerra & Yaylayan, 2012; X. Guo et al., 2018; Y. J. Ma et al., 2022; Mei et al., 2007; Scalone et al., 2015; Van Lancker et al., 2012; F. Wang et al., 2021). However, the actual temperature of red jujube is not over 100 ^{o}C during the freeze drying and the red jujube is in solid-state. Therefore, in order to more accurately study the formation of pyrazine during the freeze drying of red jujube, this study intends to establish a solid-state model system.

In addition, metabolomics technology could be used to identify amino acids, organic acids, fatty acids and sugars, which are precursors of volatile compounds, by untargeted or targeted comprehensive metabolite analysis based on spectrometry (MS) or non-MS techniques (Fu et al., 2022). It is also to predict potential metabolic pathways based on the conversion of metabolites, which is an efficient way to characterize the variation in volatile compounds in red jujube during processing. The

HPLC-MS/MS was applied to illustrate the difference in non-volatile metabolites of red jujube. The results illustrateed organic oxygen compounds, as well as lipids and lipid-like molecules, were the most up- and down-regulated metabolites in all processed red jujube, indicating carbohydrates, some carbonyl compounds, as well as lipids were the major precursors of metabolites and mainly involved in lipid oxidation and Maillard reactions. Combined with the KEGG database, differential metabolites were identified and metabolic pathways in each treatment were significantly enriched, the potential formation pathways of key aromas in red jujube after different treatments were predicted.

1.4 Connection between the different chapters of aroma research in red jujube

In order to guide the production of freeze-dried red jujube and achieve the purpose of aroma design, the key aroma compounds in red jujube were identified and glycosidically bound volatile compounds were detected firstly, to had a comprehensive insight of aroma composition in raw material. Then the key aroma of freeze-dried red jujube was identified to understand the impact of freeze drying on the aroma of red jujube. Since the multi-stage and variable-temperature procedure of freeze drying was applied in this study, which had a high temperature of heating plate (85-65 °C) and the maximum temperature of red jujube is close to 65 °C, therefore, chemical and enzymatic reactions occured and resulted in the different and new aroma. In addition, red jujube is rich in precursor substances, including reduce sugars, amino acids and fatty acids, which provide a substances basis for above mentioned reactions. In order to further understand the impact of freeze-drying on the aroma of red jujube, the changes in aroma compounds, aroma precursors and related enzyme activities during the freeze-drying process were monitored and their correlations were analysed. Combining the results of first three chapters, it was found that alkypyrazine compounds played an important role before and after freeze-drying of red jujube, and through correlation analysis, related precursors were initially screened. Therefore, in Chapter 4, a solid-state model system of red jujube matrix was established, and the formation pathway of alkylpyrazine was speculated based on actual production conditions. And the aroma of freeze-dried red jujube is changed by adding precursor substances to the matrix. To further understand the impact of processing methods on the aroma of red jujube, improve the processing technology of red jujube, and provide a theoretical basis for improving the aroma of

processed red jujube, the impact of other typical processing methods (steaming, baking and frying) on aroma of red jujube were also studied, and the formation pathway of their characteristic aroma was inferred through untargeted metabolomics.

2. General conclusion

Red jujube could be used not only as a fruit, but also as a Chinese folk medicine, which has high nutritional value and pleasant aroma. Currently, most of the research on the aroma of red jujube focused on the analysis of cultivars and regional differences, but lacks the study of its key aroma. In addition, freeze-dried red jujube as a favoured leisure food, its freeze-dried procedure is quite different from the laboratory scale one, in which chemical and enzyme reaction occurs to contribute a new aroma to red jujube. To improve the aroma of red jujube, a comprehensive understanding of the aroma of red jujube before and after processing was carried out.

In order to have a comprehensive understanding of the aroma of "Huizao", the key aroma-active compounds were identified based on molecular sensory science technology, including 3-hydroxy-2-butanone, butane-2,3-dione, methyl decanoate, ethyl decanoate, methyl dodecanoate, 5-propyloxolan-2-one, 5-butyloxolan-2-one, 2-ethyl-3,5-dimethyl-pyrazine, (*E*)-but-2-enoic acid, hexanoic acid, hexanal, 6-methyl-5-hepten-2-one, 3-oxobutan-2-yl acetate, 5-ethyloxolan-2-one, and 3-methyl-butanoic acid. They contributed the creamy, fruity, sweety, floral, nutty, green, sour notes to "Huizao". In addition, some key aroma compounds also detected in bound fraction, this result illustrated the aroma intensity of red jujube can be effectively enhanced by enzymatic hydrolysis.

After pilot scale freeze drying, only 26.71% of the content of aroma compounds was lost, and the loss rate was much lower than previously reported laboratory-scale freeze-dried results. In addition, 3-hydroxybutan-2-one, 3-oxobutan-2-yl acetate, ethyl heptanoate, methyl decanoate, ethyl decanoate, methyl dodecanoate, 2-ethyl-3,5-dimethyl-pyrazine, hexyl acetate, 3-methyl-butanoic acid, (*E*)-but-2-enoic acid, 5-propyloxolan-2-one, 2,3-butanediol, ethyl dodecanoate and 5-heptyloxolan-2-one were identified as key aroma compounds in freeze-dried red jujube. And 2-ethyl-3,5-dimethyl-pyrazine dominated the roasty note of aroma profile in freeze-dried jujube. After other typical processings (frying, baking and steaming), the aroma profile of red jujube has changed a lot. Aldehydes increased obviously and dominated a fatty aroma characteristic of fried red jujube, pyrazines content increased and contributed

the roasty notes of baked red jujube. In addition, the creamy and sour notes decreased after all processing treatments. Different processing methods contribute red jujube different aromas due to different temperatures and media. Freeze-drying is the processing method most similar to the aroma of original red jujube.

To investigated the reason of aroma changes after pilot scale freeze drying, the aroma precursors and related enzyme activity of red jujube were studied. Besides, the correlation among them also be analysed. Through the results, glucose and free amino acids involved in non-enzymatic reactions, they had the main correlation with the formation of esters, pyrazines and furfural; and the fatty acids and LOX involved in lipid oxidation reactions, they had the main correlation with the formation of alcohols, aldehydes and lactones. In addition, the influence degree of factors on the aroma formation of freeze-dried jujube was ranked as temperature > enzyme activity > fatty acids > amino acids. The multi-stage and variable-temperature procedure of freeze drying enhanced lipid pyrolysis reaction and non-enzymatic reaction efficiency, which can significantly improve the aroma of red jujube.

In this study, 3,5-EDMP played an important role in raw, freeze-dried and baked red jujube. In order to investigate the formation pathway of alkylpyrazine, the solid-state model based on red jujube matrix was established. This experiment showed in the red jujube matix, the dominant formation of 3,5-EDMP is condensation of 2-aminopentan-3-one produced by pentane-2,3-dione and aminoacetone produced by the Strecker degradation of methylglyoxal to form dihydropyrazine, which is then oxidised to form 3,5-EDMP. A verification experiment in a real model system illustrated that the aroma of processed red jujube products can be modulated by adding relevant aroma precursors.

3. Perspectives

The thesis used advanced analysis techniques and theories of volatile compounds to identify the key aroma of red jujube, and determined the glycosidically bound volatile compounds in red jujube by using enzymatic hydrolysis, which filling the gap of previous work focusing only on the description of aroma and identification of free volatile compounds in red jujube, and had a comprehensive understanding of the aroma of red jujube. Enzymatic hydrolysis can be used to release glycosidically bound volatile compounds to enhance the aroma quality of the products in the subsequent actual production. In addition, the freeze-dried condition of actual

production in the industry were applied in this thesis based on a pilot scale freeze-dried equipment, which was quite different with the laboratory freeze-dried equipment, and closer to the actual production. The key aroma of freeze-dried red jujube was also identified, and the aroma compounds, aroma precursors and related enzyme activities were tracked during the freeze-dried process to understand the evolution regulation and formation pathway of aroma of red jujube during the freeze drying. The results could provide a theoretical basis for improving the freeze-drying process and the quality of the product. Otherwise, alkylpyrazine played an important role in the aroma of freeze-dried red jujube, therefore, unlike the liquid matrix in the past researches, this study speculated on the formation pathway of alkylpyrazines based on the solid matrix of red jujube. Aroma precursors were also added into the matrix of red jujube to modify the aroma of freeze-dried red jujube, which provided ideas and research basis for the aroma design of future products. Finally, the formation pathways of characteristic aroma compounds produced by other typical processing methods of red jujube were speculated using untargeted metabolomics.

Extraction is the first step in aroma analysis. Most aroma compounds are lost during the extraction process step, therefore, continuous search for effective extraction method is continually being sought. Currently, solid-phase extraction, liquid-liquid extraction， solvent-assisted flavor evaporation (SAFE), stir bar sorptive extraction (SBSE), head space sampling (HS) and supercritical fluid extraction (SFE) are the most important and common methods reported in the literature on volatile compounds extraction of food. SPME is a simple, fast and efficient sample preparation method. It could integrate sampling, extraction, concentration and sample injection into one step. It is currently the most widely used extraction method (Zhang et al., 2016). Therefore, SPME method has been used to extract the volatile compounds of red jujube in each chapter of this thesis.

At present, gas chromatography (GC) is the main method for separating volatile compounds for its high sensitivity and good separation ability. GC separates the mixture mainly accords the polarity, boiling point, and adsorption properties of the sample. Since volatile compounds are composed of multiple compounds, different compounds have different polarities. Usually, both polar and non-polar chromatographic columns are used to extract the aroma comprehensively. The existence of chiral molecules is also a very interesting phenomenon in aroma research. Most enantiomer pairs have different odors (such as R and S limonene)

(Genva et al., 2019). Most of the identified compounds in red jujube were acids, esters, aldehydes and ketones based on the literature reported on red jujube, and most of these compounds are polar compounds. For this reason, polar wax been used to separate the volatile compounds of red jujube in each chapter of this thesis.

In order to study the aroma compounds of red jujube and its products in greater depth, a number of suggestions were made: (1) use a variety of extraction methods to extract compounds, use non-polar chromatographic columns to separate red jujube and its products, and use more sophisticated instruments, such as GC×GC or chiral chromatography columns, to better separate compounds and comprehensive understand the composition of red jujube aroma. (2) To obtain a broader production of volatile compounds from glycosidically bound volatile compounds, the AR2000 or glycosidase could be used. (3) During the freeze drying, a large amount of cold trap water will be produced, which also contains some volatile compounds. The cold trap water can be utilized to extract aroma compounds or make drinking water. (4) Due to limited time, it is recommended that the isotope labelling be used to study the formation pathyway of key aroma during the processing of red jujube in next research. (5) The formation of aroma is a complex process, involving not only chemical and enzymatic reactions, but also the physical interaction between aroma and matrix and aroma, which has inhibition or release effects, and ultimately forms the aroma of the product. Therefore, it is necessary to study the interaction between aroma and matrix and between aromas in order to better understand the formation of aroma.

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Appendix – publications

1. Articles

(1) **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**, 39(06), 3066-3079. https://doi.org/10.1080/87559129.2021.2005088

(2) **Gou, M**., Chen, Q., Qiao, Y., Li, J., Long, J., Wu, X., Zhang, J., Fauconnier, M.-L, Jin, X., Lyu, J., & Bi, J. (**2022**). Comprehensive investigation on free and glycosidically bound volatile compounds in Ziziphus jujube cv. Huizao. Journal of **Food Composition and Analysis**, 112(March), 104665. https://doi.org/10.1016/j.jfca.2022.104665

(3) **Gou, M**., Chen, Q., Qiao, Y., Jin, X., Zhang, J., Yang, H., Fauconnier, M. -L., & Bi, J. (**2023**). Key aroma-active compounds identification of Ziziphus jujuba cv. Huizao: Effect of pilot scale freeze-drying. **Journal of Food Composition and Analysis**, 116(November 2022), 105072. https://doi.org/10.1016/j.jfca.2022.105072

(4) **Gou, M**., Chen, Q., Wu, X., Liu, G., Fauconnier, M.-L., & Bi, J. (**2023**). Novel insight into the evolution of volatile compounds during dynamic freeze-drying of Ziziphus jujuba cv. Huizao based on GC–MS combined with multivariate data analysis. **Food Chemistry**, 410, 135368. https://doi.org/10.1016/j.foodchem.2022.135368

(5) **Gou, M**., Bi, J., Liu, G., Fauconnier, M.-L., & Chen, Q.. Characterization of key aroma-active compounds changes and formation in red jujube as affected by different processing methods via GC-MS/MS and untargeted. **Prepare to submit**.

(6) Modelling alkylpyrazine formation in red jujube matrix under controlled conditions of pH and temperature. Food Chemistry. Submitted to journal.