Journal of Food Composition and Analysis

Identification and formation of key aroma-active compounds in red jujube as affected by different processing methods

--Manuscript Draft--

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	Qinqin Chen
Abstract:	Processing methods significantly affect the aroma characteristics of red jujube; however, few studies have explored how. Gas chromatography-tandem mass spectrometric detection, gas chromatography olfactometry, relative odour activity value and quantitative descriptive analysis were used to investigate the aroma changes of red jujube during typical processing (freeze drying, baking, frying and steaming); furthermore, the formation of aroma-active compounds in processed red jujube was predicted based on untargeted metabolomics. 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. In addition, ethyl esters dominated the sweet and fruity notes in freeze-dried red jujube, which were formed via combination of fatty acids metabolism, pyruvate metabolism and amino acid catabolism. (E, E)-Deca-2,4-dienal dominated the fatty note in fried red jujube, which were the products of fatty acid oxidation. 2-Ethyl-3,5-dimethylpyrazine dominated the roasty notes of baked red jujube due to the Maillard reaction.
Suggested Reviewers:	Yuyu Zhang Beijing Technology and Business University zhangyuyu@btbu.edu.cn
	Parker Jane K University of Reading j.k.parker@reading.ac.uk
Response to Reviewers:	Response to Reviewer Comments Dear Stephen Elmore, Your Ref: “Identification and formation of key aroma-active compounds in red jujube as affected by different processing methods”, which you submitted to Journal of Food Composition and Analysis by Gou et al. (Manuscript ID: JFCA-D-24-01724) Thank you for your email message of 17/07/2024. My co-authors and I would also like to thank the reviewers the valuable comments to improve our manuscript. We have now carefully considered the comments and have made corrections, we hope will meet with approval. Revised portions are marked in red in the manuscript. The corrections and the response to the editor and reviewers’ comments are as follows: Editor Comments: Please double-check those identifications not confirmed with standards. Some of them seem unlikely. Response: Thanks very much for your guidance. We have double-checked those identified compounds not confirmed with standards. In this study, the qualitative methods of the compounds mainly included linear retention index (LRI), MS

spectrometry, standards and GC-O. Those compounds that were not identified by standards were identified by mass spectrometry and comparison of linear retention index with those in the literature. And the determined LRI by n-alkanes and LRI in the literature have been provided in Table 1.
Reviewers' comments:
Reviewer #1：
Abstract.
1. Line 25: Authors should provide the full meaning of acronyms (GC-MS,MS, GC-Oetc.,) at the first usage
Response: Thanks for your kind reminder. The full meaning of acronyms (GC-MS/MS, GC-O, rOAV) has been provided in “Abstract”.
Introduction,
2. Line 63. .... extraction and "qualified" should read quantified
Response: Thanks for your kind reminder. The “qualified” has been modified to “quantified” in line 65.
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3. Lines 115 -116: Authors should provide a detailed explanation for their processes with necessary references
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4. Line 116: What is the ratio of jujube to oil?
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5. Lines 156-157: The so called corresponding compounds should be listed along with their respective aroma.
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6. Line 163. Delete "presented"and replace with represent
Response: Thanks for your kind reminder. The “presentes” has been modified to “represent in line 186.
7. Line 167. What samples are you referring to?
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8. Lines 169 -172. Authors should provide appropriate references for this method
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Results.
9.Lines 277-280. Authors should provide an in-depth discussion for the significant increase in the concentration of aldehydes. Discussion in lines 403-413would have been appropriate here.
Response: Thanks very much for your guidance. The in-depth discussion for the significant increase in the concentration of aldehydes has been added in lines 306-318. In this manuscript, the changes in the content of aroma compounds are discussed in detail in the section “3.2. Effect of different processing methods on the aroma compounds in red jujube”, while the reasons for the changes in the content of aroma compounds are discussed in-depth in the section “3.5. Potential transformation pathways of key odour-active compounds after different treatments”.
10. Lines 329-330: "human nose was used" This is wrong expression -Authors should replace with sniffing evaluation was performed.
Response: Thanks for your kind reminder. The “human nose” has been replaced into the sniffing evaluation” in line 361.
Conclusions
11. Authors failed to articulate the significance of their study to science.
Response: Thanks very much for your guidance. The significance of this study to science has been provided in lines 496-499.
Reviewer #2:
This manuscript (JFCA-D-24-01724): Identification and formation of key aroma-active compounds in red jujube as affected by different processing methods. The topic is interesting. However, there are some critical flaws as follows:
1. 2.2. Sample preparation：the preparation methods seems unreasonable, especially for the frying. Usually, the frying temperature is lower than 180 ℃, but in this study, the frying temperature was 210 ℃.
Response: Thanks very much for your guidance. Generally lower frying temperatures

	are accompanied by longer frying times. In this study, although the frying temperature was 210 °C, the frying process lasted only 10 s. In some literatures, the frying temperature was also more than 180 °C. Such as, Hu et al. (2022) deep-fired the grass carps at the temperature of 200 °C for 2-8 min; and Wei et al. (2023) fired the yellow croaker at the temperature of 220 °C for 1.5 min. References: Hu, X. fei, Li, J. lin, Zhang, L., Wang, H., Peng, B., Hu, Y. ming, Liang, Q. xi, Tu, Z. cai, 2022. Effect of frying on the lipid oxidation and volatile substances in grass carp (Ctenopharyngodon idellus) fillet. J. Food Process. Preserv. 46. https://doi.org/10.1111/JFPP.16342 Wei, H., Wei, Y., Qiu, X., Yang, S., Chen, F., Ni, H., Li, Q., 2023. Comparison of potent odorants in raw and cooked mildly salted large yellow croaker using odor-active value calculation and omission test: Understanding the role of cooking method. Food Chem. 402, 134015. https://doi.org/10.1016/j.foodchem.2022.134015 2. 2.4. Headspace solid-phase microextraction (HS-SPME): lots of factors can affect the release of volatiles in sample and the extraction effect of HS-SPME, such as moisture and oil content, structures of the samples, etc, but in this study, the authors omitted all those factors which were totally different among the samples. Therefore, I don't think those quantification data in Table 1 and conclusion are reasonable. Response: Thanks very much for your guidance. Indeed, texture, matrix and water content will affect the release of aroma compound. If different extraction methods are used, it is more complicated to compare the compounds between different samples. Therefore, the same extraction conditions by HS-SPME of different samples were used in this study. When do the comparison analysis among different treated samples, the same extraction method was usually applied. Such as, Wei et al. (2023) used HS-SPME quantified the volatile content of steamed-, baked, fried- and deep fried yellow croakers. According to your constructive suggestions, the release of volatiles as affected by matrix, moisture content, structures of samples as well as extraction methods would be investigated in our future study. Many thanks. Reference: Wei, H., Wei, Y., Qiu, X., Yang, S., Chen, F., Ni, H., Li, Q., 2023. Comparison of potent odorants in raw and cooked mildly salted large yellow croaker using odor-active value calculation and omission test: Understanding the role of cooking method. Food Chem. 402, 134015. https://doi.org/10.1016/j.foodchem.2022.134015 3. The results lack of novelty, as it's a common sense that aldehydes increased obviously and dominated a fatty aroma characteristic of fried red jujube, and pyrazines content increased and contributed the roasty notes of baked red jujube, and freeze-dried red jujube had a similar aroma profile to raw red jujube. Response: Thanks very much for your guidance. In this study, not only the contribution of compound categories to the aroma of different processed red jujube was investigated, but also the aroma-active compounds produced by red jujube after different processing methods were identified by GC-O and rOAV. For example, (E, E)-2, 4-deca-dienal and 2-ethyl-3,5-dimethylpyrazine contributed most to the aroma of fried red jujube, and in baked red jujube, 2-ethyl-3,5-dimethylpyrazine mainly contributed to the roasty note. Methyl dodecanoate, ethyl heptanoate and methyl decanoate contributed the most to the aroma of freeze-dried red jujube. Finally, non-targeted metabolomics was used to speculate on the formation pathway of these aroma-active compounds that are formed after processing. With this study, we hope to provide a data basis for future regulation of the aroma of red jujube processed products. 4. 3.4. Untargeted metabolome by HPLC-MS/MS: "as well as lipids were the major precursors of metabolites" and conclusion "These changes were the results of the interaction of fatty acid metabolism and the Maillard reaction.", I cannot agree that the oxidation and degradation of fatty acid equals to fatty acid metabolism in this study. Response: Thanks very much for your guidance. Metabolites and fatty acid metabolism are broad concepts. In this study, we only focused on differential metabolites, the title of this section has been changed to “3.4. Differential metabolites analysis” (line 397). In addition, lipids are important precursor of volatile metabolites in this study, this sentence has also been changed to “lipids were the major precursors of volatile compounds” (line 410); fatty acid oxidation and degradation is only part of the fatty acid metabolism, to be more rigorous, we also revised the sentence “These changes were the results of the interaction of fatty acid oxidation/degradation and the Maillard reaction” (line 494). Thank you for your constructive guidance to let our article more rigorous.

Reviewer #3:
The research paper "Identification and formation of key aroma-active compounds in red jujube as affected by different processing methods" provides clear insights about the impact of processing methods on the aroma of red jujube. The manuscript is well structured and interesting, even if a revision of English language is strongly recommended. The paper needs to undergo several modifications to enhance its readability and quality upon its publication at Journal of Food Composition and Analysis.
1. Line 87- How much time were the samples stored at 4 °C? This is a crucial point in terms of preservation and possible fermentation of the matrices, as they were not frozen.
Response: Thanks for your kind reminder. In this study, red jujube was used in the experiment within 3 months of harvest. Generally, red jujube can be stored for more than one year under the condition of 4 oC. Since the water content of red jujube is only 25.57% and has thicker skin, red jujube will not be quality deterioration and fermentation. And the corresponding information has been supplemented in line 90.
2. lines 114-115 - Steamed red jujube were obtained in a covered steamer for 10 minutes.
Response: Thanks for your kind reminder. This sentence has been corrected in line 119.
3. lines 119, 122,134, 149 - The reference Gou et al (2023) is cited, is it 2023a or b?
Response: Thanks for your kind reminder. The format of the reference has been improved (line 124, 133, 147, 172, 246, 248 and 478).
4. section 2.4 please briefly describe the extraction method.
Response: Thanks very much for your guidance. The detailed extraction method has been provided in lines 147-155.
5. lines 153-154 - why all these aroma descriptors are not reported in Figure 1A?
Response: Thanks very much for your guidance. Figure 1 (a) is the result of sensory evaluation. Its corresponding aroma descriptors are in the section “2.3. Sensory evaluation” (lines 135-138). And the aroma descriptors in lines 176-178 are mainly used to train the results of smelling through GC-O, they are reported in Table 1.
6. lines 188-189 - please motivate the choice to use Arb units
Response: Thanks for your kind reminder. The unit “Arb” has been replaced by “mL/min” in line 212.
7. line 196 - Please explain CV
Response: Thanks for your kind reminder. The CV referred to coefficient of variance, the full meaning of acronyms (CV) has been provided in line 222.
8. line 196 - Authors need to explain how do they manage the total database of metabolites. In untargeted metabolites analysis, the data are processed by a software that reveals the annotated compounds with different scores according to the reliability of the results and it match with data libraries. For this purpose, the authors must provide with score threshold do they choose and which parameters do they apply to reach the final total list of compound that went through statistics.
Response: Thanks very much for your guidance. When screening metabolites, we mainly use the score of primary mass spectrometry and the fragmentation score of secondary mass spectrometry to screen metabolites. Since each substance peak in the qualitative process can represent multiple metabolites, the higher the score, the higher the accuracy of metabolite identification. In the screening process, we did not set a threshold for the score. If the scores were the same, mass error (ppm) and isotope similarity were used to assist in the screening of metabolites. The smaller the mass error, the smaller the deviation between the measured mass-to-charge ratio and the theoretical value and the higher the accuracy of metabolite identification. The closer the isotope similarity is to 1, the more similar the determined isotope distribution pattern is to the theoretical model thereby increasing the reliability of metabolite identification. The corresponding parameters of screening metabolites has been added to lines 217-219.
9. Table 1 - The use of internal standard (as reported in lines 143-144) is useful to monitor the extraction performances, not for quantification of analytes. According to this, only the molecules confirmed with standards could be reported with concentration unit (ug/Kg). The others needs to be reported as area percentage as they were not quantified. Moreover two columns need to be added to report the calculated RI and the literature RI of each compound.
Response: Thanks very much for your guidance. In this study, all volatile compounds were quantified using a semi-quantitative method. To clearly different from μg/kg, the

concentrations have been expressed as μg/kg of 2-octanol equivalents. The corresponding instructions have been added to the line 166 and Table 1. And the LRI determined by n-alkanes and those found in literature also have been added in Table 1.
10. lines 272-275 and 300-302 report the same concept.
Response: Thanks very much for your guidance. Lines 301-302 and 330-333 illustrated the decrease in acids and alcohols contents, respectively. Acids and some alcohols have higher vapor pressures resulted they are all more likely to volatilize. To avoid misunderstanding, the sentences have been modified (lines 330-333).
11. lines 285-288 - As the compound discussed were reported in different fried matrices, these are strictly related to frying oil. Please remark.
Response: Thanks for your kind reminder. Frying oil not only serves as a heating medium in the frying process, but also provides a rich source of fatty acids for the formation of product aroma. The frying oil used in the different matrices has been marked and discussed their role in aroma formation (lines 310-318).
12. line 296 - decreased significantly in other processed red jujube.
Response: Thanks for your kind reminder. This sentence has been corrected in line 326.
13. Figure 2 caption is not correct. Figure 2A is not up- and down- regulated metabolites and 2B is not a KEGG enrichment plot. Moreover the Steaming vs raw KEGG pathway is reported with letter D but it must be F. Please check and modify.
Response: Thanks for your kind reminder. The caption of Figure 2 has been modified.
14. Figure 2B - colors are not clear
Response: Thanks for your kind reminder. The colors of Figure 2 (b) have been changed.

Fig. 2 (b) The numbers of up- and down-regulated metabolites.
15. In KEGG pathways, the p-values (ad so the red-yellow colors) to which of the compared groups are referred? higher or lower p-values means that a pathway is enriched in which of the 2 groups? Please explain in the figure or in the caption.
Response: Thanks very much for your guidance. The higher the value on the horizontal axis, the higher the enrichment of differential metabolites in the KEGG pathway. The color of the point represents the p-value of the hypergeometric test. The smaller the value, the greater the reliability of the test and the higher the statistical significance. The size of the point represents the number of differential metabolites in the corresponding KEGG pathway. The larger the value, the more differential metabolites there are in the KEGG pathway. In this study, the four KEGG enrichment maps were compared with raw red jujube, and the results were the KEGG enrichment of (c) freeze-dried red jujube, (d) baked red jujube, (e) fried red jujube, and (f) steamed red jujube. The corresponding explanation has been added to Figure caption.
16. In general, the discussion is mainly focused on high-temperature modifications that can occur in baking and steaming processes. In my opinion, also the low-temperature and low-pressure conditions of freeze-drying have a crucial impact on volatiles, which could be furtherly discussed.
Response: Thanks very much for your guidance. Different from the laboratory scale freeze dryer, pilot scale freeze dryer was used in this study, and the detailed freeze-drying parameters have been supplemented in the section “2.2. Sample preparation” (lines 123-130). During the pilot scale freeze drying, the temperature of the sample will gradually rise from -40 oC to 65 oC. According to our previous study, the aroma changes of red jujube during the pilot scale freeze drying mainly started from the 165 min, when the temperature has reached 57 oC (Gou et al., 2023). Therefore, high temperature is also one of the factors leading to the formation of freeze-dried red jujube aroma.
Reference:
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 Chem. 410, 135368. https://doi.org/10.1016/j.foodchem.2022.135368
Reviewer #4:
The Manuscript describes the identification and formation of aroma-active compounds

	in red jujube as result of different processing methods. The work is also enriched by untargeted metabolomic approach. However, there are some issues that need to be addressed before publication. 1. The highlights should briefly present the work and attract the attention of the readers. In this case, the authors did not even present red jujube as object of the study. For this reason, the highlights should be re-organized to allow readers to understand the topic and the main results achieved. Response: Thanks very much for your guidance. The highlights have been reorganized as follows: The changes of key aroma of red jujube after different treatments were studied. Formation pathway of key aroma in red jujube via different treatments was predicted. Fatty acid oxidation and Maillard reaction led to key aroma changes in red jujubes. 2. There are some typos all over the manuscript, see for instance: Line 52. Remove full stop before the brackets. Line 70. Substitute 'of' with 'for'. Line 129. Correct 'panellist'. Line 134. Remove comma after Gou. Line 152. Remove comma after 'al.'. Line 161-163. Control margins for this paragraph. Line 169-170. Please correct and rephrase this sentence. Line 214-215. Please rephrase this sentence. Line 220, 246, . Remove comma after Gou. Line 222. Remove comma after 'al.'. Response: Thanks for your kind reminder. We have checked and corrected typos and reference format throughout the manuscript and marked them in red (line 54, 57, 72, 140, 147, 184, 195, 246, 228). The sentences in lines 192-193 and lines 240-241 have also been rewritten and marked in red. 3. Uniform citation of Tables into the text. Should they be in bold or not? Response: Thanks for your kind reminder. The citation of Tables in this manuscript has been uniformed and marked them in red. 4. The scientific name of the plant should be accompanied by the relative authorship the first time it is cited into the text (line 85). Response: Thanks for your kind reminder. We have revised this section, the scientific name of the plant first appeared in the “Introduction” and accompanied by the relative authorship (lines 42-44). 5. Table 1 should report also the RI found in literature to assign the compound. Response: Thanks very much for your guidance. The LRI determined by n-alkanes and those found in literature have been added in Table 1. We are grateful to Journal of Food Composition and Analysis for giving us the chance to revise our work. We appreciate your and the reviewers’ warm work earnestly and hope that the corrections will meet with approval. We are looking forward to your response. Yours sincerely, Jinfeng Bi

Cover letter

Dear Editor,

Here within enclosed is our paper for consideration to be published on ***Journal of Food Composition and Analysis***, the title is "***Identification and formation of key aroma-active compounds in red jujube as affected by different processing methods***". Red jujube as a resource both used as foods and traditional Chinese medicine, which is rich in nutrition and has a pleasant flavour, and consumers prefer processed red jujube products. Due to the influence of different temperatures, heat transfer methods and heating media in the process, red jujube will undergo various chemical reactions, resulting in significant differences in the aroma and quality of the products. Processing methods significantly affect the aroma characteristics of red jujube; however, few studies have explored how. GC-MS/MS, GC-O, rOAV and quantitative descriptive analysis were used to investigate the aroma changes of red jujube during typical processing (freeze drying, baking, frying and steaming); furthermore, the formation of aroma-active compounds in processed red jujube was predicted based on untargeted metabolomics. 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. In addition, esters dominated the sweet and fruity notes in freeze-dried red jujube, 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 red jujube, which were the products of lipid oxidation and fatty acid metabolism. Pyrazines dominated the roasty notes of baked red jujube due to the Maillard reaction

All the authors agree to the submission of this paper to the journal.

Thank you very much for consideration!

Yours Sincerely,

Jinfeng Bi

Response to Reviewer Comments

Dear Stephen Elmore,

Your Ref: "Identification and formation of key aroma-active compounds in red jujube as affected by different processing methods", which you submitted to Journal of Food Composition and Analysis by Gou et al. (Manuscript ID: JFCA-D-24-01724)

Thank you for your email message of 17/07/2024. My co-authors and I would also like to thank the reviewers the valuable comments to improve our manuscript. We have now carefully considered the comments and have made corrections, we hope will meet with approval. Revised portions are marked in red in the manuscript. The corrections and the response to the editor and reviewers' comments are as follows:

Editor Comments:

Please double-check those identifications not confirmed with standards. Some of them seem unlikely.

Response: Thanks very much for your guidance. We have double-checked those identified compounds not confirmed with standards. In this study, the qualitative methods of the compounds mainly included linear retention index (LRI), MS spectrometry, standards and GC-O. Those compounds that were not identified by standards were identified by mass spectrometry and comparison of linear retention index with those in the literature. And the determined LRI by *n*-alkanes and LRI in the literature have been provided in Table 1.

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1. Line 25: Authors should provide the full meaning of acronyms (GC-MS,MS, GC-Oetc.,) at the first usage

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The research paper "Identification and formation of key aroma-active compounds in red jujube as affected by different processing methods" provides clear insights about the impact of processing methods on the aroma of red jujube. The manuscript is well structured and interesting, even if a revision of English language is strongly recommended. The paper needs to undergo several modifications to enhance its readability and quality upon its publication at Journal of Food Composition and Analysis.

1. Line 87- How much time were the samples stored at 4 °C? This is a crucial point in terms of preservation and possible fermentation of the matrices, as they were not frozen.

Response: Thanks for your kind reminder. In this study, red jujube was used in the experiment within 3 months of harvest. Generally, red jujube can be stored for more than one year under the condition of 4 ºC. Since the water content of red jujube is only 25.57% and has thicker skin, red jujube will not be quality deterioration and

fermentation. And the corresponding information has been supplemented in line 90.

2. lines 114-115 - Steamed red jujube were obtained in a covered steamer for 10 minutes.

Response: Thanks for your kind reminder. This sentence has been corrected in line 119.

3. lines 119, 122,134, 149 - The reference Gou et al (2023) is cited, is it 2023a or b?

Response: Thanks for your kind reminder. The format of the reference has been improved (line 124, 133, 147, 172, 246, 248 and 478).

4. section 2.4 please briefly describe the extraction method.

Response: Thanks very much for your guidance. The detailed extraction method has been provided in lines 147-155.

5. lines 153-154 - why all these aroma descriptors are not reported in Figure 1A?

Response: Thanks very much for your guidance. Figure 1 (a) is the result of sensory evaluation. Its corresponding aroma descriptors are in the section "2.3. Sensory evaluation" (lines 135-138). And the aroma descriptors in lines 176-178 are mainly used to train the results of smelling through GC-O, they are reported in Table 1.

6. lines 188-189 - please motivate the choice to use Arb units

Response: Thanks for your kind reminder. The unit "Arb" has been replaced by "mL/min" in line 212.

7. line 196 - Please explain CV

Response: Thanks for your kind reminder. The CV referred to coefficient of variance, the full meaning of acronyms (CV) has been provided in line 222.

8. line 196 - Authors need to explain how do they manage the total database of metabolites. In untargeted metabolites analysis, the data are processed by a software

that reveals the annotated compounds with different scores according to the reliability of the results and it match with data libraries. For this purpose, the authors must provide with score threshold do they choose and which parameters do they apply to reach the final total list of compound that went through statistics.

Response: Thanks very much for your guidance. When screening metabolites, we mainly use the score of primary mass spectrometry and the fragmentation score of secondary mass spectrometry to screen metabolites. Since each substance peak in the qualitative process can represent multiple metabolites, the higher the score, the higher the accuracy of metabolite identification. In the screening process, we did not set a threshold for the score. If the scores were the same, mass error (ppm) and isotope similarity were used to assist in the screening of metabolites. The smaller the mass error, the smaller the deviation between the measured mass-to-charge ratio and the theoretical value and the higher the accuracy of metabolite identification. The closer the isotope similarity is to 1, the more similar the determined isotope distribution pattern is to the theoretical model thereby increasing the reliability of metabolite identification. The corresponding parameters of screening metabolites has been added to lines 217-219.

9. Table 1 - The use of internal standard (as reported in lines 143-144) is useful to monitor the extraction performances, not for quantification of analytes. According to this, only the molecules confirmed with standards could be reported with concentration unit (ug/Kg). The others needs to be reported as area percentage as they were not quantified. Moreover two columns need to be added to report the calculated RI and the literature RI of each compound.

Response: Thanks very much for your guidance. In this study, all volatile compounds were quantified using a semi-quantitative method. To clearly different from μg/kg, the concentrations have been expressed as μg/kg of 2-octanol equivalents. The corresponding instructions have been added to the line 166 and Table 1. And the LRI determined by n-alkanes and those found in literature also have been added in Table 1.

10. lines 272-275 and 300-302 report the same concept.

Response: Thanks very much for your guidance. Lines 301-302 and 330-333 illustrated the decrease in acids and alcohols contents, respectively. Acids and some alcohols have higher vapor pressures resulted they are all more likely to volatilize. To avoid misunderstanding, the sentences have been modified (lines 330-333).

11. lines 285-288 - As the compound discussed were reported in different fried matrices, these are strictly related to frying oil. Please remark.

Response: Thanks for your kind reminder. Frying oil not only serves as a heating medium in the frying process, but also provides a rich source of fatty acids for the formation of product aroma. The frying oil used in the different matrices has been marked and discussed their role in aroma formation (lines 310-318).

12. line 296 - decreased significantly in other processed red jujube.

Response: Thanks for your kind reminder. This sentence has been corrected in line 326.

13. Figure 2 caption is not correct. Figure 2A is not up- and down- regulated metabolites and 2B is not a KEGG enrichment plot. Moreover the Steaming vs raw KEGG pathway is reported with letter D but it must be F. Please check and modify.

Response: Thanks for your kind reminder. The caption of Figure 2 has been modified.

14. Figure 2B - colors are not clear

Response: Thanks for your kind reminder. The colors of Figure 2 (b) have been changed.

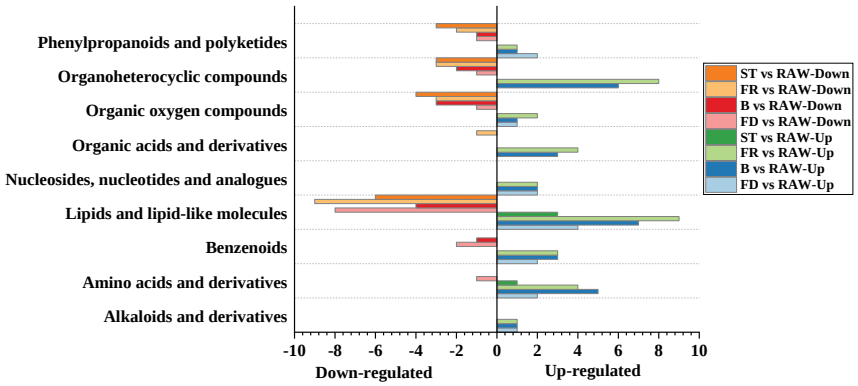

Fig. 2 (b) The numbers of up- and down-regulated metabolites.

15. In KEGG pathways, the p-values (ad so the red-yellow colors) to which of the compared groups are referred? higher or lower p-values means that a pathway is enriched in which of the 2 groups? Please explain in the figure or in the caption.

Response: Thanks very much for your guidance. The higher the value on the horizontal axis, the higher the enrichment of differential metabolites in the KEGG pathway. The color of the point represents the p-value of the hypergeometric test. The smaller the value, the greater the reliability of the test and the higher the statistical significance. The size of the point represents the number of differential metabolites in the corresponding KEGG pathway. The larger the value, the more differential metabolites there are in the KEGG pathway. In this study, the four KEGG enrichment maps were compared with raw red jujube, and the results were the KEGG enrichment of (c) freeze-dried red jujube, (d) baked red jujube, (e) fried red jujube, and (f) steamed red jujube. The corresponding explanation has been added to Figure caption.

16. In general, the discussion is mainly focused on high-temperature modifications that

can occur in baking and steaming processes. In my opinion, also the low-temperature and low-pressure conditions of freeze-drying have a crucial impact on volatiles, which could be furtherly discussed.

Response: Thanks very much for your guidance. Different from the laboratory scale freeze dryer, pilot scale freeze dryer was used in this study, and the detailed freeze-drying parameters have been supplemented in the section "2.2. Sample preparation" (lines 123-130). During the pilot scale freeze drying, the temperature of the sample will gradually rise from -40 ºC to 65 ºC. According to our previous study, the aroma changes of red jujube during the pilot scale freeze drying mainly started from the 165 min, when the temperature has reached 57 ºC (Gou et al., 2023). Therefore, high temperature is also one of the factors leading to the formation of freeze-dried red jujube aroma.

Reference:

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 Chem. 410, 135368. https://doi.org/10.1016/j.foodchem.2022.135368

Reviewer #4:

The Manuscript describes the identification and formation of aroma-active compounds in red jujube as result of different processing methods. The work is also enriched by untargeted metabolomic approach. However, there are some issues that need to be addressed before publication.

1. The highlights should briefly present the work and attract the attention of the readers. In this case, the authors did not even present red jujube as object of the study. For this

reason, the highlights should be re-organized to allow readers to understand the topic and the main results achieved.

Response: Thanks very much for your guidance. The highlights have been reorganized as follows:

- The changes of key aroma of red jujube after different treatments were studied.
- Formation pathway of key aroma in red jujube via different treatments was predicted.
- Fatty acid oxidation and Maillard reaction led to key aroma changes in red jujubes.

2. There are some typos all over the manuscript, see for instance:

Line 52. Remove full stop before the brackets.

Line 70. Substitute 'of' with 'for'.

Line 129. Correct 'panellist'.

Line 134. Remove comma after Gou.

Line 152. Remove comma after 'al.'.

Line 161-163. Control margins for this paragraph.

Line 169-170. Please correct and rephrase this sentence.

Line 214-215. Please rephrase this sentence.

Line 220, 246, . Remove comma after Gou.

Line 222. Remove comma after 'al.'.

Response: Thanks for your kind reminder. We have checked and corrected typos and reference format throughout the manuscript and marked them in red (line 54, 57, 72, 140, 147, 184, 195, 246, 228). The sentences in lines 192-193 and lines 240-241 have

also been rewritten and marked in red.

3. Uniform citation of Tables into the text. Should they be in bold or not?

Response: Thanks for your kind reminder. The citation of Tables in this manuscript has been uniformed and marked them in red.

4. The scientific name of the plant should be accompanied by the relative authorship the first time it is cited into the text (line 85).

Response: Thanks for your kind reminder. We have revised this section, the scientific name of the plant first appeared in the “Introduction” and accompanied by the relative authorship (lines 42-44).

5. Table 1 should report also the RI found in literature to assign the compound.

Response: Thanks very much for your guidance. The LRI determined by n-alkanes and those found in literature have been added in Table 1.

We are grateful to ***Journal of Food Composition and Analysis*** for giving us the chance to revise our work. We appreciate your and the reviewers’ warm work earnestly and hope that the corrections will meet with approval.

We are looking forward to your response.

Yours sincerely,

Jinfeng Bi

Highlights

- The changes of key aroma of red jujube after different treatments were studied.
- Formation pathway of key aroma in red jujube via different treatments was predicted.
- Fatty acid oxidation and Maillard reaction led to key aroma changes in red jujubes.

Identification and formation of key aroma-active compounds in red jujube as affected by different processing methods

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Abstract:

Processing methods significantly affect the aroma characteristics of red jujube; however, few studies have explored how. Gas chromatography-tandem mass spectrometric detection, gas chromatography olfactometry, relative odour activity value and quantitative descriptive analysis were used to investigate the aroma changes of red jujube during typical processing (freeze drying, baking, frying and steaming); furthermore, the formation of aroma-active compounds in processed red jujube was predicted based on untargeted metabolomics. 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. In addition, ethyl esters dominated the sweet and fruity notes in freeze-dried red jujube, which were formed via combination of fatty acids metabolism, pyruvate metabolism and amino acid catabolism. (*E*, *E*)-Deca-2,4-dienal dominated the fatty note in fried red jujube, which were the products of fatty acid oxidation. 2-Ethyl-3,5-dimethylpyrazine dominated the roasty notes of baked red jujube due to the Maillard reaction.

Keywords: freeze drying, frying, steaming, baking, GC-MS/MS, 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, and "Huizao" is one of the most popular varieties of red jujube (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) is superior for retaining the colour, shape, texture and nutritional value of red jujubes, and is 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., 2023b). 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 analyzed based on extraction and quantified 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 for 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/MS and relative odour activity value (rOAV). 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 °C until use within 3 months.

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. For steaming, 500 g red jujube were placed in a covered steamer with a perforated divider for 10 min (Wei et al., 2023). For frying, 200 g red jujube were put in corn oil (red jujube: oil = 1:10 (g/mL)) preheated to 210 °C for 10 seconds. For baking, 500 g red jujube were put on the baking pan of an oven (PT 2531, Media Co., Ltd., Anhui, China) at 180 °C for 5 min (Qiao et al., 2022a). For freeze drying, the conditions are described in Gou et al. (2023a). Briefly, 500 g jujube slices put into -40 °C refrigerator for 48 h. A pilot scale freeze dryer (Advantech Co., Ltd. China) was used and drying parameters were as follows: The cold trap temperature, -30 °C; vacuum pressure, 60 Pa; the temperature program of the heating plate: room temperature 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.

2.3. Sensory evaluation

Sensory evaluation method is described in Gou et al. (2021) and Gou et al. (2023a). Briefly, 10 panellists with experience in quantitative descriptive analysis (QDA) formed the sensory panel. In order to evaluate the odour description and intensity, standard solutions of 3-hydroxybutan-2-one (creamy), (*E*, *E*)-2, 4-decadienal (fatty), hexanal (green), methyl dodecanoate (fruity), 2,6-dimethylpyrazine (roasty), (*E*)-but-2-enoic acid (sweet), acetic acid (sour), 2-ethyl-6-methylpyrazine (nutty) and furaneol (caramel) were dissolved in water (containing 5% methanol) and final diluted to 100 times of its odor threshold. These samples were put in 20 mL vials and randomly numbered. Panellist 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 (2 cm, Supelco, Inc., Bellefonte, PA, USA) were used in this study. The extraction conditions for volatile components of different samples by HS-SPME were described by Gou et al. (2023b) and Gou et al. (2022). Briefly, after removing the kernel, red jujube 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 and 0.1 g NaCl in 0.5 mL of distilled water were placed in 20 mL headspace vials, and 2 μL of

2-cyclo-hexen-1-one (1 mg/mL) which was used as internal standard was then added. Subsequently, the samples were incubated at 50 °C for 40 min, then the SPME fiber was adsorbed and extracted at 50 °C 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. The experiment was repeated at least three times independently for each sample.

2.5. GC-MS/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. 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). The results were expressed as μg/kg of 2-cyclohexene-1-one equivalents.

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., 2023a).

GC-O experiments were performed by 3 trained panelists (three women, aged

from 23-27 years). Before the formal experiment, the panelists need to undergo daily training. The training method referred to Niu et al. (2021), including correctly identifying the aroma of different compounds and their intensity. Aroma types mainly involved mushroom, fruity, corn flakes, caramel, green, grass, fatty, roasty, almond, berry, baked, butter, creamy, sweet, sweat, sour, floral, putrid fruit, rancid, coconut and nutty. The corresponding standard compounds were diluted into different concentrations (10 ppm - 640 ppm) allowed the panelists could be familiar with the aroma intensity. In the formal GC-O experiment, the sample would be sniffed once by each of three panelists.

2.7. Relative odour activity value (rOAV)

The rOAV was determined according to Liu et al. (2024), which was as follows:

$rOAV=C_i/OT_i$

where: C_i represent the concentration of the compound, and OT_i represent 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 raw and all processed red jujube, involved baked-, fried-, freeze dried- and steamed- red jujube were frozen by liquid nitrogen and ground into a powder with a Joyoung pulveriser (JYL-CO20, Joyoung Co., Ltd., Shandong, China). Put 100 mg of powder into 500 µL lysate (MeOH: H_2O = 1:1), vortex for 30 seconds, and keep overnight at -20 °C. Then centrifuged at 13,000 rpm for 20 min at 4 °C, collected the supernatant, and 100 µL was drawn into an injection

bottle (Liu et al., 2024).

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 was 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 ^{o}C, 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 L/min; the flow of auxiliary gas and sweep gas was 55 L/min; 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 score of primary mass sepctrometry, fragmentation score of secondary mass sepctrometry, mass error (ppm) and isotope similarity were mainly used to screen metabolites. 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 coefficient of variance (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 processing were evaluated through quantitative descriptive sensory analysis (QDA), and the results are displayed in **Fig. 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. The aroma profile of raw red jujube is related to sweet, fruity, green, creamy, sour and nutty notes, which is consistent with 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., 2023b). By contrast, freeze-dried samples showed similar intensities of sweet and fruity notes with raw samples, which was in line with Gou et al. (2023a). There were two group in the clustered heatmap (**Fig. 1(b)**), one group is raw, steamed and freeze-dried samples, the other 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.

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 1**). The different processing methods had significant effects on the volatiles. 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

261 was increased to 56 compounds. Furthermore, to compare the contents of volatile
262 compounds in different processed red jujube, the relative contents of volatile
263 compounds were calculated by internal standard. The total volatile content was
264 dramatically decreased after steaming (78.74%), frying (74.11%) and baking
265 (51.60%), but increased slightly after freeze drying (0.90%) (**Table 1**).

266 Esters were the most abundant volatile compounds in the raw red jujube (15,332
267 μg/kg), which contributed to fruity and sweet notes (**Table 1 and Table 2**). However,
268 for fried red jujube, the total content of esters declined sharply (97.42%), followed by
269 steamed red jujube (84.20%) and baked red jujube (45.00%). In contrast to the other
270 treatments, the esters increased by 45.90% after freeze drying (**Fig.1 (c)**), which was
271 attributed to non-enzymatic esterification of alcohols and organic acids, amino acid
272 metabolism and fatty acid oxidation, this result was also in agreement with our
273 previous studies (Gou et al., 2023a; Gou et al., 2023b). Among these esters,
274 methyl decanoate, ethyl decanoate and methyl dodecanoate were key aroma-active
275 compounds of red jujube (Gou et al., 2022), which dominated the fruity, sweet and
276 creamy notes of raw red jujube. The contents of these three compounds significantly
277 decreased or were no longer detectable in baked, fried and steamed red jujube, while
278 increased in freeze-dried red jujube. In addition, four ester compounds detected only
279 in freeze-dried red jujube, namely ethyl octanoate, ethyl dodecanoate, methyl
280 (Z)-tetradec-9-enoate and methyl (Z)-hexadec-9-enoate. Especially, ethyl dodecanoate,
281 with sweet and fruity notes, was identified as the key aroma compound in freeze-dried
282 red jujube (Gou et al., 2023a). Therefore, the changes in content of esters are the main

283 reason for the differences in sweet and fruity notes in different processed red jujube.
284 Similar to esters, lactones also provide sweet and fruity notes to raw red jujube. And
285 5-propyloxolan-2-one, 5-butyloxolan-2-one and 5-ethyloxolan-2-one were key aroma
286 compounds in raw red jujube (Gou et al., 2022). After different processing, the
287 contents of these lactone compounds reduced or no longer detected (**Fig.1 (c)**), which
288 was another reason why the sweet and fruity notes of red jujube were weakened after
289 freeze drying, baking, frying and steaming.

290 As another main volatile compounds in red jujube, the total contents of acids
291 decreased significantly, especially after steaming (81.03%), followed by frying
292 (77.59%), baking (75.15%) and freeze drying (56.48%) (**Fig.1 (c)**). Among these
293 acids, hexanoic acid, 3-methyl-butanoic acid and (*E*)-but-2-enoic acid were key aroma
294 compounds in red jujube, with sour notes (Gou et al., 2023a). Therefore, a significant
295 decrease in these acids content resulted in weaker or no perception of sour notes in the
296 processed red jujube. Notably, acetic acid as the acid compound with the highest
297 content in raw red jujube, was also reduced significantly, especially after steaming
298 (83.52%), followed by baking (79.18%), frying (63.91) and freeze drying (40.12%)
299 (**Table 1**). The reduction of acid compounds may be due to their participation in
300 chemical reactions during heat processing, such as Maillard reaction and esterification.
301 In addition, most acid compounds have higher vapor pressures and are more likely to
302 volatilize under high temperature conditions.

303 The content of aldehydes in red jujube decreased after freeze drying, but
304 increased significantly after other treatments, especially after frying, leading to the

changes in sensory characteristics. The aldehyde content in fried red jujube was 3.2 times that of the original red jujube (**Fig.1 (c)**). Some new aldehydes, which with fatty notes, were detected in processed red jujube. For example, (*E*, *E*)-deca-2,4-dienal, (*E*, *E*)-hepta-2,4-dienal, nonanal, furfural, (*E*)-oct-2-enal and (*E*)-dec-2-enal were detected only in fried red jujube, which might be produced by lipid oxidation and fatty acid degradation. These compounds were also found in fried chicken breast meat with soybean oil (Zhang et al., 2018), fried mountain pepper oil (Ni et al., 2021), French fries with soybean oil (Xu et al., 2022), and fried yellow croaker with corn oil (Wei et al., 2023), and identified as the characteristic aroma compounds of frying. Frying oil not only serves as a heating medium in the frying process, but also provides a rich source of fatty acids for the formation of product aroma. Soybean oil is rich in linoleic and linolenic acids, corn oil is also a rich source of oleic acid and linoleic acid. These fatty acids can be degraded or oxidized during the heating process and form aldehyde compounds, leading to an increase in aldehyde content. In addition, the decreased hexanal content caused the green note to disappear, and the increased contents of (*E, E*)-deca-2,4-dienal and (*E, E*)-hepta-2,4-dienal contributed to the fatty note in fried red jujube. Changes in the types and contents of aldehydes resulted the change in the aroma of processed red jujube. Especially for fried red jujube, the green note caused by aldehydes disappeared and transformed to a fatty note caused by aldehydes.

Alcohols, also as products of fatty acid degradation, increased significantly in fried red jujube (133.51%) and decreased significantly in other processed red jujube

(**Fig.1 (c)**). Corn oil was used as the medium during the frying process of red jujube. And corn oil contains rich unsaturated fatty acids, especially oleic acid and linoleic acid, so it can provide a sufficient material basis to produce fatty alcohols and fatty aldehydes in fried red jujube. However, it is noteworthy that alcohols were no longer detected after steaming, which could be due to a thermally catalysed synthesis. Or, like acids, some short-chain alcohols also have a higher vapour pressure and are more likely to volatilize (Wei et al., 2023).

Pyrazines contributed a nutty note to raw red jujube, and 2-ethyl-3,5-dimethyl-pyrazine identified as a key aroma compounds in red jujube (Gou et al., 2022). In addition to decreased after steaming, the pyrazines content in red jujube increased after freeze drying, frying, and baking. Especially in baked red jujube, the pyrazines content increased more than two-fold that of the raw red jujube (**Fig.1 (c)**), hence, roasty was changed as the dominant aroma in baked red jujube. And for freeze-dried red jujube and fried red jujube, the nutty note caused by pyrazines also changed to a roasty note (**Fig. 1(a)**). Generally, the production of pyrazines mainly depends on the Maillard reaction, which occurs under high-temperature conditions during processing. In addition, some heterocyclic compounds and sulphur-containing compounds, such as 2-ethylfuran, 1-(furan-2-yl)ethenone, 1-nitropyrrolidine, 1-nitroso-2,5-dihydropyrrole, 1-(4H-Pyridin-1-yl)ethanone and (5-methylfuran-2-yl) methanol, which are all products of the Maillard reaction, can contribute to the roasty notes in fried or baked red jujube.

A total of 10 ketones were detected in all samples, among which

butane-2,3-dione, 6-methyl-5-hepten-2-one and 3-hydroxybutan-2-one, with creamy notes, were identified as the key aroma compounds in red jujube (Gou et al., 2022). The contents of these ketone compounds in red jujube decreased after processing, resulting in a weakening of the creamy note in the processed red jujube. Furthermore, 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, and eventually pyrazines and other heterocyclic compounds would be formed (Deng et al., 2022; Yu et al., 2021). This also explains the reason for the changes in the content of ketones, acids and pyrazines after processing.

3.3. GC-O and rOAV analysis

In this study, the rOAV was applied to assess the contribution of odour active volatile compounds to the overall aroma of different samples. Additionally, the sniffing evaluation was used as a detector in combination with GC to identify volatile compounds that influence the overall aroma of the samples and to determine the intensity of these aroma compounds. From **Table 2**, there were 23, 18, 11, 13 and 9 aroma-active compounds of raw, freeze-dried, baked, fried and steamed red jujube, respectively (rOAV $\geq$ 1). And there were 26, 29, 21, 26 and 17 aroma compounds perceived by GC-O in raw, freeze-dried, baked, fried and steamed red jujube, respectively. Because the relevant thresholds were not found for some volatile compounds, resulting in missing rOAV data, further leading to the difference results between GC-O and rOAV.

From **Table 2**, the GC-O results showed that strong intensity of sweet, fruity,

371 sour, creamy and nutty were frequently occurring aroma descriptors of raw red jujube,
372 and these aroma descriptors correspond to esters, acids, ketones and pyrazines.
373 Similarly, sweet, fruity, sour and creamy were also mainly aroma descriptors of
374 freeze-dried red jujube. But the aroma intensity changed a lot compared to raw red
375 jujube. In freeze-dried red jujube, the intensity of the sour and creamy notes decreased,
376 while the intensity of sweet and fruity notes increased. There were also more ester
377 compounds perceived as fruity and sweet notes in the freeze-dried red jujube. For
378 steamed red jujube, sweet, nutty were mainly aroma descriptors, and almost aroma
379 compounds presented the weak intensity. For baked red jujube, caramel and roasty
380 were the frequently occurring aroma descriptors, which mainly related to pyrazines
381 and heterocyclic compounds. Aldehydes with the strong or middle fatty notes and
382 pyrazines with strong or middle roasty notes were frequently perceived in fried red
383 jujube. These results agreed with the sensory evaluation results **(Fig. 1(a)**).

384 A Sankey diagram was employed to visualize the results of rOAV (**Fig. 1(d)**). It
385 was observed that esters, especially methyl dodecanoate (rOAV=2933), ethyl
386 heptanoate (rOAV = 1426) and methyl decanoate (rOAV = 1324) had the higher rOAV
387 in freeze-dried red jujube. Besides, the rOAV of 3,5-EDMP (1468) was markedly
388 increased after freeze drying, which resulted the changes in the aroma profile. (*E, E*)-2,
389 4-Deca-dienal (rOAV = 1752) and 3,5-EDMP (rOAV = 1712) were the dominant
390 contributors to the aroma of fried red jujube, and 3,5-EDMP had the highest rOAV
391 (2706) in the baked red jujube. It was noted that 3,5-EDMP had the aroma
392 contribution to all five samples, but the aroma characteristic was unique to each

sample (**Table 2**). 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. Differential metabolites analysis

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 (8.28%), benzenoids (7.01%), organoheterocyclic compounds (14.65%), organic acids and derivatives (5.1%), organic oxygen compounds (9.55%), lipids and lipid-like molecules (31.85%), alkaloids and their derivatives (1.91%), nucleosides, nucleotides and their analogues (3.82%), phenylpropanoids and polyketides (7.01%) and others (10.83%) (**Fig.2(a)**). From **Fig.2(b)**, 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 volatile compounds and mainly involved in lipid oxidation and Maillard reactions.

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 ($p < 0.05$, **Fig. 2(c)**). In baked red jujube, the metabolism of fatty acid, α-linolenic acid, tyrosine, arachidonic acid, purine and isoquinoline alkaloid biosynthesis were obviously enhanced ($p < 0.05$, **Fig. 2(d)**), whereas in the fried and steamed samples, only biosynthesis of unsaturated fatty acids and fatty acid biosynthesis, respectively, were significantly enriched ($p < 0.05$, **Fig. 2(e)(f)**). In general, fatty acids had a significant influence on aroma formation in red jujube during different treatments.

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

Aldehydes, esters and pyrazines were the main compounds responsible for the difference in aroma profile among fried, freeze-dried and baked red jujube and raw red jujube. By comparison with the KEGG database, differential metabolites were identified and metabolic pathways in each treatment were significantly enriched, the potential formation pathways of aldehydes, esters and pyrazines in red jujube after different processing were predicted (**Fig. 3**).

Aldehydes, especially nonanal, 2-pentylfuran, (*E*)-hept-2-enal, (*E*, *E*)-deca-2,4-dienal and (*E*, *E*)-hepta-2,4-dienal, which had fatty notes, dominated the aroma profile of fried red jujube. The fatty acids, such as oleic acid, linoleic acid and α-linolenic acid from red jujube and oleic acid and linoleic acid from corn oil, can be subjected to auto/enzyme oxidation to form various hydroperoxides (n-ROOH) (Du et al., 2020; Hidalgo and Zamora, 2019), such as 10-ROOH and 11-ROOH, and their carbon-carbon bonds can be broken by alkoxy α/β-cleavage to form nonanal and

decanal (**Fig. 3**). Nonanal was only detected in fried red jujube and contributed a fatty note to fried sample. Besides, (*E, E*)-hepta-2,4-dienal, (*E, E*)-deca-2,4-dienal and (*E*)-oct-2-enal were also aroma-active compounds in fried red jujube, which produced from *α*-linolenic. *α*-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 (Hu et al., 2022; Sohail et al., 2022). In addition, alkyl furans, such as 2-pentylfuran and 2-propylfuran, are mainly generated from linoleic acid (Frank et al., 2020). 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 (**Fig. 3**).

Esters, with fruity and sweet notes, had a significant contribution to aroma profiles in freeze-dried and steamed red jujube. The content of esters, especially that of ethyl esters, included ethyl heptanoate, ethyl decanoate, ethyl dodecanoate, ethyl hexanoate and ethyl octanoate, with fruity and sweet notes, 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 (**Fig. 3**). Additionally, acyl-CoA could be generated from fatty acid oxidation and amino acid metabolism (Schwab et al., 2008).

Pyrazines are key aroma compounds of baked red jujube, and contributed the roasty note. It is worth mentioning that 3,5-EDMP made a significant contribution to the aroma in all five samples. In addition, DDMP (3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one) with caramel and roasty notes were perceived and detected in baked, fried and steamed red jujube. 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 (**Fig. 3**). 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 (**Fig. 3**). Furthermore, glucose, sucrose and fructose, the three main sugars in red jujube (Gou, et al., 2023b), 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 (**Fig. 3**). 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. Ester content increased significantly and contributed to the fruity and sweet notes for freeze-dried red jujube. In addition, the creamy and sour notes decreased after all processing treatments. These changes were the results of the interaction of fatty acid oxidation/degradation and the Maillard reaction. Lastly, freeze-dried and steamed red jujube had a similar aroma profile to raw red jujube, which could have a positive effect on overall aroma acceptance. Through investigating the effects of different processing methods on the aroma characteristics of red jujube and the formation of the key aromas of processed red jujube products, we could provide the scientific support for the aroma regulation of red jujube products in the future study.

Ethical approval

The study followed the recommendations of the Declaration of Helsinki and was in accordance with the China regulations concerning research involving human volunteers (Notice on Issuing the Measures for Ethical Review of Life Sciences and

Medical Research Involving Humans). All panellists participated voluntarily and provided written consent after being informed about the aims and potential risks of the study and consented to the publication of relevant data.

CRediT authorship contribution statement

Min Gou: Conceptualization, Data curation, Formal analysis, Methodology, Investigation, Writing-original draft & editing. **Jinfeng Bi:** Conceptualization, Resources, Supervision, Writing-review & editing. **Gege Liu:** Investigation. **Marie-Laure Fauconnier**: Supervision, Writing-review & editing. **Qinqin Chen:** Conceptualization, Supervision, Writing-review & editing, Methodology, Validation.

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Conflict of Interest

The authors have declared no conflict of interest.

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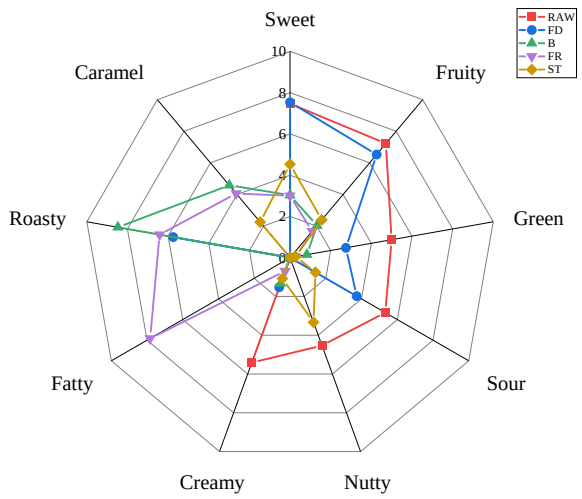
Figure Captions:

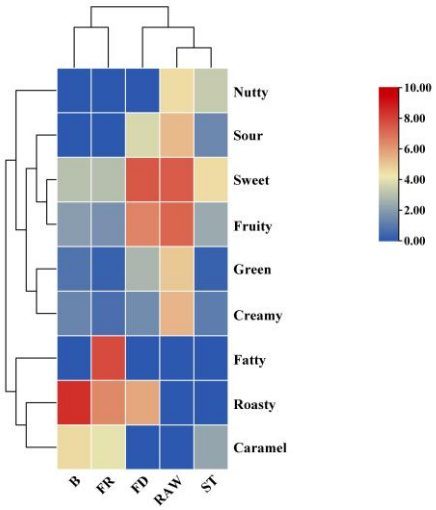
Fig. 1 Sensory evaluation results based on quantitative descriptive analysis (QDA) (a) and the Hierarchical clustering heatmap plot (b); concentration of different types of aroma compounds in different processed red jujube (d) Sankey diagram based on rOAV values of all processed red jujube.

Fig. 2 (a) The classification and percentage of the key differential metabolites; (b) the numbers of up- and down-regulated metabolites; the four KEGG enrichment maps were compared with raw red jujube, and the results were the KEGG enrichment of (c) freeze-dried red jujube, (d) baked red jujube, (e) fried red jujube, and (f) steamed red jujube (The higher the value on the horizontal axis, the higher the enrichment of differential metabolites in the KEGG pathway. The color of the point represents the p-value of the hypergeometric test. The smaller the value, the greater the reliability of the test and the higher the statistical significance. The size of the point represents the number of differential metabolites in the corresponding KEGG pathway. The larger the value, the more differential metabolites there are in the KEGG pathway).

Fig. 3 The sketch map of potential source of the aroma active compounds in different processed red jujube based on KEGG and reference. The pink, sky blue, yellow and green colour represented the aroma active compounds in baked, fried, freeze-dried and steamed red jujube, respectively. The dark blue colour represented the aroma active compounds in all processed red jujube.



(a)



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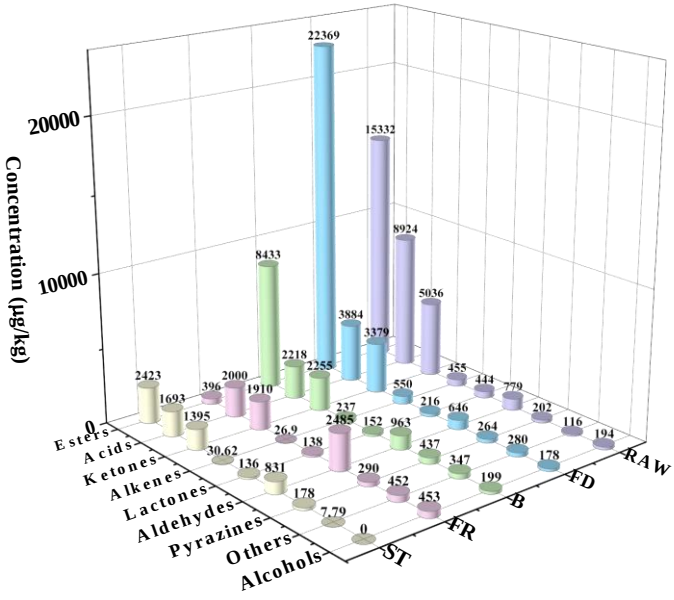

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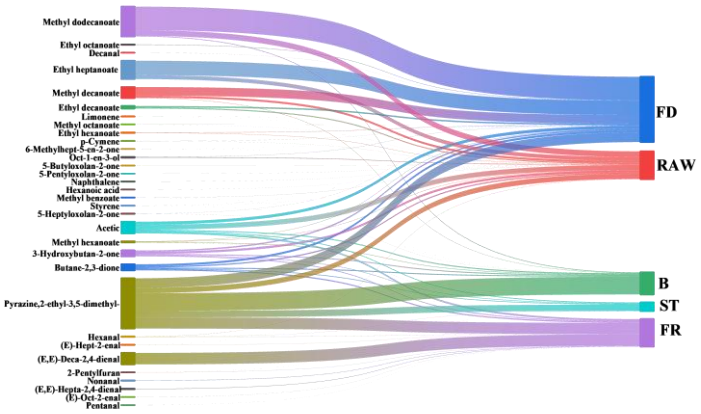

(d)

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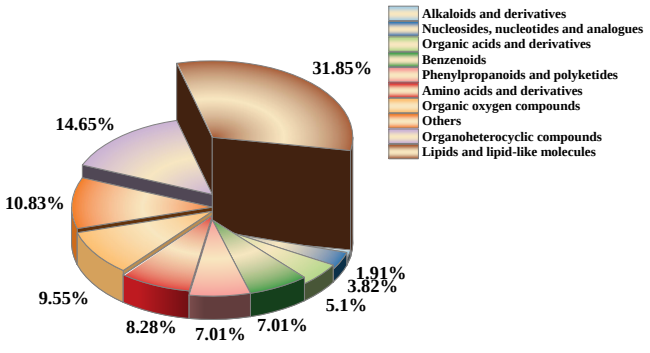

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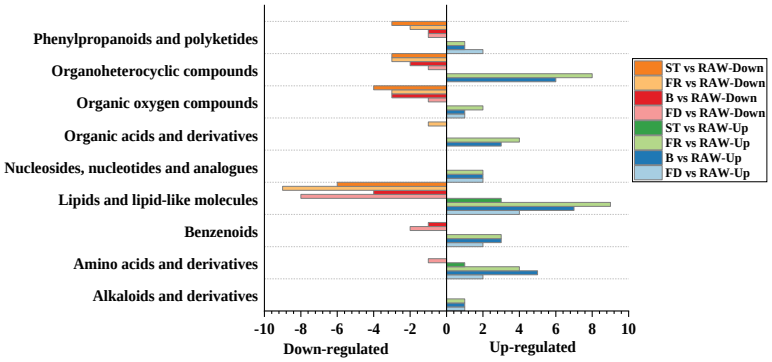

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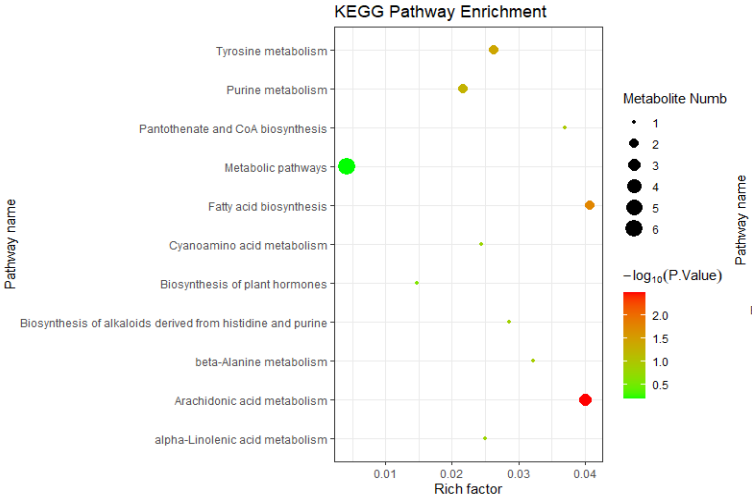
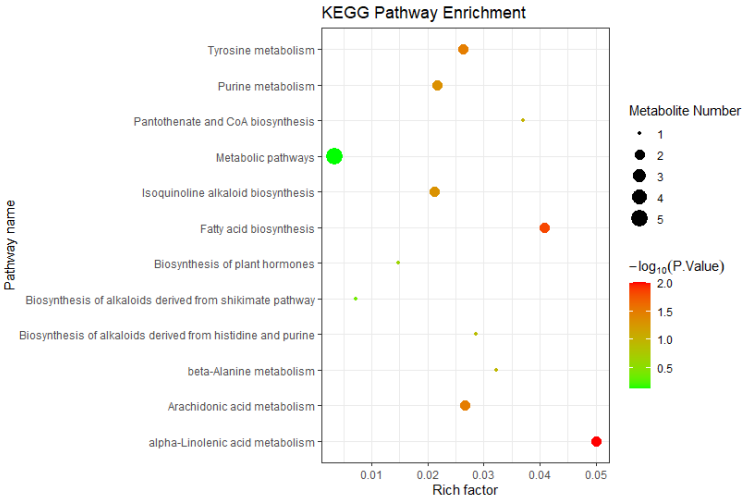
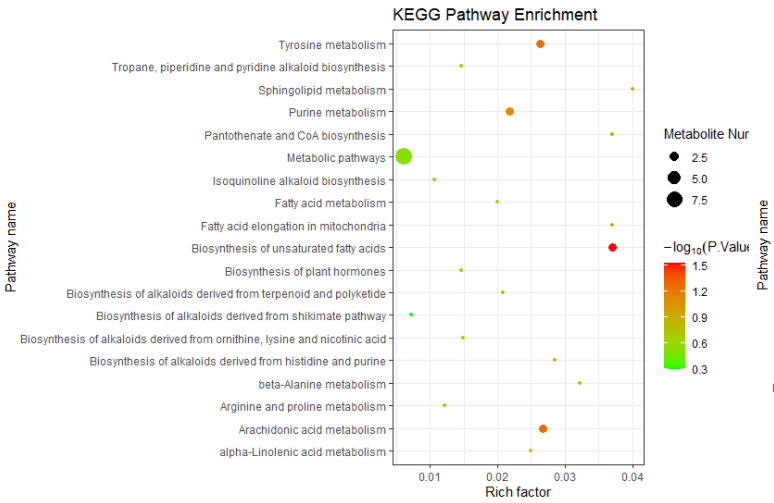


(c) Freeze drying vs Raw



(d) Baking vs Raw



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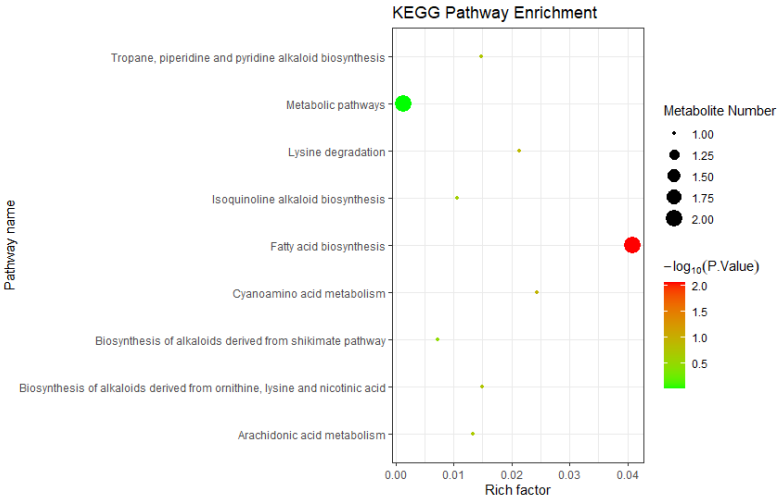

(f) Steaming vs Raw

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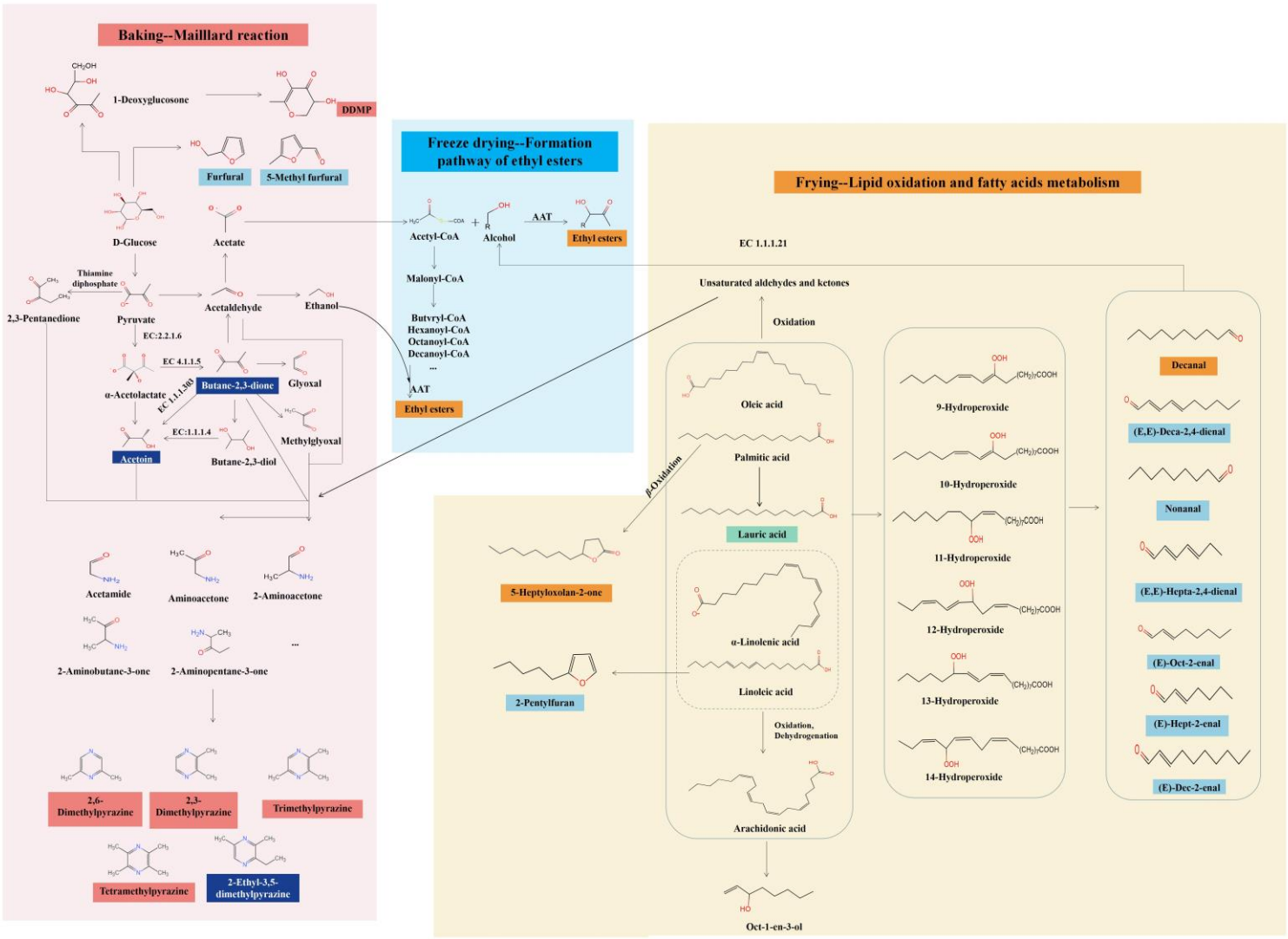

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Table 1. Volatile compounds identified in red jujube samples from different treatments.

Code	LRI[a/b]	Compounds	Identification	Concentration (μg/kg of 2-cyclohexene-1-one)[c]				
				RAW	FD	B	FR	ST
		Alcohols						0
A1	1543/1553	Butane-2,3-diol	MS, LRI, Std	97.33±13.83ab	113±1a	79.15±4.55b	52.42±2.79c	n.d
A2	1665/1669	Furan-2-ylmethanol	MS, LRI	n.d	n.d	97.54±5.83b	229±2a	n.d
A3	1679/1684	Furan-3-ylmethanol	MS, LRI	n.d	n.d	22.39±3.09b	149±8a	n.d
A4	1729/1720	(5-Methylfuran-2-yl)methanol	MS, LRI	n.d	n.d	n.d	22.79±0.74	n.d
A5	1450/1448	Oct-1-en-3-ol	MS, LRI, O, Std	96.57±4.84a	28.37±3.98b	n.d	n.d	n.d
A6	1855/1853	2-Butyloctan-1-ol	MS, LRI	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, LRI	n.d	n.d	122±12b	167±2a	n.d
B2	890/880	2-Methylbutanal	MS, LRI	n.d	n.d	54.92±1.4	n.d	n.d
B3	900/932	3-Methylbutanal	MS, LRI, O, Std	n.d	99.21±11.61b	235±16a	213±1a	114±8b
B4	960/945	2-Ethylfuran	MS, LRI, O	n.d	n.d	n.d	7.16±0.47	n.d
B5	980/974	Pentanal	MS, LRI	n.d	n.d	n.d	38.96±0.89	n.d
B6	1014/1043	2-Propylfuran	MS, LRI, Std	n.d	n.d	n.d	n.d	2.59±0.09
B7	1083/1078	Hexanal	MS, LRI, O, Std	106±13a	n.d	n.d	89.37±1.98b	81.64±2.11b
B8	1216/1228	(*E*)-Hex-2-enal	MS, LRI, Std	39.61±3.36a	n.d	n.d	32.82±0.3b	17.79±1.56c
B9	1230/1249	2-Pentylfuran	MS, LRI, 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, LRI, O	n.d	n.d	n.d	123±2	n.d

B11	1291/1287	Octanal	MS, LRI, 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, LRI, Std	16.93±3.32b	n.d	n.d	202±5a	n.d
B13	1392/1409	Nonanal	MS, LRI, O, Std	n.d	n.d	n.d	63.07±0.82	n.d
B14	1429/1410	(*E*)-Oct-2-enal	MS, LRI, Std	n.d	n.d	n.d	31.58±2.14	n.d
B15	1463/1479	Furfural	MS, LRI, O, Std	89.09±8.82c	329±4b	308±1b	1054±34a	397±14b
B16	1497/1451	(*E*,*E*)-Hepta-2,4-dienal	MS, LRI, O	n.d	n.d	n.d	139±5	n.d
B17	1519/1508	Benzaldehyde	MS, LRI, O, Std	487±18a	197±12b	182±1b	69.69±3.76c	205±29b
B18	1520/1498	Decanal	MS, LRI, Std	n.d	21.63±0.67	n.d	n.d	n.d
B19	1540/1615	(*E*)-Dec-2-enal	MS, LRI	n.d	n.d	n.d	65.19±3.72	n.d
B20	1597/1582	5-Methyl furfural	MS, LRI, 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, LRI	n.d	n.d	30.18±2.95	n.d	n.d
C2	979/979	Butane-2,3-dione	MS, LRI, O, Std	187±1b	320±12a	144±3c	172±4b	101±7d
C3	1061/1062	Pentane-2,3-dione	MS, LRI, Std	n.d	n.d	46.53±0.41a	25.48±0.68b	n.d
C4	1286/1286	3-Hydroxybutan-2-one	MS, LRI, O, Std	4698±72a	2910±218b	2003±23c	1680±18cd	1294±128d
C5	1339/1342	6-Methylhept-5-en-2-one	MS, LRI, Std	102±10a	74.08±11.24b	n.d	n.d	n.d
C6	1378/-	3-Oxobutan-2-yl acetate	MS, LRI, O, Std	49.07±4.54b	74.32±2.67a	n.d	n.d	n.d
C7	1547/1535	Cyclopent-4-ene-1,3-dione	MS, LRI	n.d	n.d	8.64±0.2b	30.3±0.46a	n.d
C8	1670/1645	1-Phenylethanone	MS, LRI	n.d	n.d	4.24±1.23	n.d	n.d
C9	1780/-	3,4-Dimethylfuran-2,5-dione	MS, LRI	n.d	n.d	n.d	3.05±0.11	n.d
C10	1912/-	3-Methyl-2H-furan-5-one	MS, LRI, 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, LRI, O, Std	4217±135a	2525±225ab	878±15c	1522±26b	695±144c
D2	1528/1508	Propionic acid	MS, LRI, Std	82.62±3.47a	113±12a	110±25a	126±2a	n.d
D3	1623/1628	Butanoic acid	MS, LRI, Std	121±11.2a	102±19a	50.59±1.15b	n.d	32.78±1.63b
D4	1665/1680	3-Methylbutanoic acid	MS, LRI, O, Std	217±0b	321±8a	199±5b	97.22±2.81c	113±14c
D5	1732/1762	Pentanoic acid	MS, LRI, Std	121±11.2a	108±11a	n.d	n.d	70.71±12.37b
D6	1753/1750	(*E*)-But-2-enoic acid	MS, LRI, O, Std	44.17±2.90a	37.25±3.16b	n.d	n.d	13.82±2.84b
D7	1841/1849	Hexanoic acid	MS, LRI, O, Std	3326±219a	458±26b	605±32b	255±68c	491±7b
D8	1943/1943	Heptanoic acid	MS, LRI, Std	227±1a	n.d	224±12a	n.d	n.d
D9	2178/2171	Nonanoic acid	MS, LRI, Std	111±12a	n.d	8.15±0.85b	n.d	n.d
D10	2320/2279	Decanoic acid	MS, LRI, Std	458±10a	221±3b	142±4c	n.d	137±29c
D11	2487/2502	Dodecanoic acid	MS, LRI, 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, LRI, Std	1155±113a	414±14c	777±20b	89.00±4.23d	23.67±1.36d
E2	902/899	Methyl propionate	MS, LRI	888±19a	67.1±8.33c	202±3b	n.d	n.d
E3	945/989	Methyl butanoate	MS, LRI	1737±41a	169±16cd	857±6b	80.3±4.9d	186±13c
E4	1011/1018	Methyl 3-methylbutanoate	MS, LRI	557±4a	25.58±1.09c	230±8b	n.d	n.d
E5	1078/1082	Methyl pentanoate	MS, LRI	1406±103a	236±21cd	978±25b	72.11±7.14d	403±37c
E6	1181/1177	Methyl hexanoate	MS, LRI, Std	3201±300b	3217±212b	4754±325a	78.8±8.86d	593±55c
E7	1233/1241	Ethyl hexanoate	MS, LRI, Std	174±12b	221±8a	n.d	n.d	n.d

E8	1245/1259	Methyl (*E*)-hex-3-enoate	MS, LRI	n.d	n.d	26.04±2.2	n.d	n.d
E9	1272/1265	Hexyl acetate	MS, LRI, O, Std	324±29.30a	162±12.58b	n.d	n.d	n.d
E10	1326/1342	Ethyl heptanoate	MS, LRI, O, Std	113±9.90b	271±28.75a	n.d	n.d	n.d
E11	1372/1374	Methyl octanoate	MS, LRI, Std	748±5b	1009±82a	64.1±0.29c	n.d	n.d
E12	1457/1441	Ethyl octanoate	MS, LRI, Std	n.d	842±49	n.d	n.d	n.d
E13	1518/1536	Methyl nonanoate	MS, LRI, Std	100±2b	149±1a	n.d	n.d	n.d
E14	1593/1636	Methyl decanoate	MS, LRI, O, Std	1370±135b	5693±500a	187±5c	n.d	n.d
E15	1623/1631	Methyl benzoate	MS, LRI, Std	853±13a	60.96±6.48c	98.21±2.18b	n.d	n.d
E16	1638/1633	Ethyl decanoate	MS, LRI, O, Std	788±5a	1095±2a	n.d	n.d	n.d
E17	1804/1834	Methyl dodecanoate	MS, LRI, O, Std	1305±124b	4399±153a	154±4c	n.d	1148±80b
E18	1815/1830	Methyl cyclopentenolone	MS, LRI	n.d	n.d	n.d	75.99±8.05a	62.46±6.49a
E19	1833/1854	Methyl 3-phenylpropanoate	MS, LRI	n.d	n.d	105±2a	n.d	6.04±0.82b
E20	1841/1849	Ethyl dodecanoate	MS, LRI, O, Std	n.d	2940±4	n.d	n.d	n.d
E21	1959/-	Methyl (*Z*)-tetradec-9-enoate	MS, LRI, O	575±4b	889±61a	n.d	n.d	n.d
E22	2016/2037	Methyl tetradecanoate	MS, LRI, O, Std	n.d	380±25	n.d	n.d	n.d
E23	2201/2243	Methyl hexadecanoate	MS, LRI, Std	38.25±3.39a	43.13±4.18a	n.d	n.d	n.d
E24	2264/2277	Methyl (*Z*)-hexadec-9-enoate	MS, LRI, O	n.d	86.12±6.03	n.d	n.d	n.d
		Total		**15332**	**22369**	**8433**	**396**	**2423**
		Lactones						
F1	1108/-	4-Methyloxolan-2-one	MS, LRI	28.15±0.24a	n.d	2.97±0.29b	n.d	n.d
F2	1590/1600	5-Methyloxolan-2-one	MS, LRI	n.d	n.d	9.65±0.61	n.d	n.d
F3	1639/1602	Oxolan-2-one	MS, LRI, 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, LRI, 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, LRI, 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, LRI, O, Std	55.84±5.83	n.d	n.d	n.d	n.d
F7	2068/2096	5-Acetyloxolan-2-one	MS, LRI, O	7.7±0.77	n.d	n.d	n.d	n.d
F8	2083/2063	5-Pentyloxolan-2-one	MS, LRI, O	52.92±9	n.d	n.d	n.d	n.d
F9	2221/2238	5-Heptyloxolan-2-one	MS, LRI, Std	n.d	20.8±2.34a	15.45±0.72b	n.d	n.d
F10	2395/2384	5-Octyloxolan-2-one	MS, LRI	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, LRI	n.d	n.d	33.17±0.77b	53.23±0.58a	n.d
G2	1321/1319	2,6-Dimethylpyrazine	MS, LRI, 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, LRI, O	n.d	n.d	n.d	14.29±0.5	n.d
G4	1369/1399	2-Ethyl-5-methylpyrazine	MS, LRI, Std	n.d	n.d	32.11±3.97a	n.d	17.78±0.24b
G5	1378/1363	2-Ethyl-6-methylpyrazine	MS, LRI, O, Std	n.d	n.d	64.1±0.2a	n.d	30.76±3.66b
G6	1398/1413	Trimethylpyrazine	MS, LRI, 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, LRI, Std	n.d	n.d	n.d	5.21±0.54	n.d
G8	1452/1464	2-Ethyl-3,5-dimethyl-pyrazine	MS, LRI, 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, LRI, 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, LRI, Std	274±2b	405±38a	146±6c	9.02±0.04d	30.62±3.31d
H2	1245/1238	γ-Terpinene	MS, LRI, Std	n.d	45.94±1.18	n.d	n.d	n.d

H3	1255/1254	Styrene	MS, LRI, 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, LRI	n.d	39.38±3.62	n.d	n.d	n.d
I2	1095/1261	*p*-Cymene	MS, LRI, Std	39.42±3.15a	45.3±3.43a	n.d	n.d	n.d
I3	1102/-	2,6-Dimethyldecane	MS, LRI, O	n.d	107±2	n.d	n.d	n.d
I4	1164/1139	1,4-Xylene	MS, LRI, Std	49.34±0.46a	45.75±3.17a	39.98±1.14a	13.56±1.04b	n.d
I5	1280/1707	Naphthalene	MS, LRI, Std	26.95±3.73	n.d	n.d	n.d	n.d
I6	1394/-	1-Nitroso-2,5-dihydropyrrole	MS, LRI	n.d	n.d	n.d	31.15±0.61	n.d
I7	1512/-	1-(Furan-2-yl)ethanone	MS, LRI, O	n.d	n.d	37.22±1.71a	33.78±0.47b	n.d
I8	1740/-	Pyrazine-2-carboxamide	MS, LRI	n.d	42.38±0.1b	n.d	66.43±0.83a	n.d
I9	1780/-	1-(4H-Pyridin-1-yl)ethanone	MS, LRI, O	n.d	n.d	83.9±3.43b	99.62±0.74a	n.d
I10	1989/-	1-Nitropyrrolidine	MS, LRI	n.d	n.d	n.d	114±10	n.d
I11	2278/-	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one	MS, LRI, 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**

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. MS, identified by MS spectra; O, identified by comparison of their odor description with the authentic compounds via GC-O; Std, identified by comparison to standards.

[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 relative contents of volatile compounds were expressed as μg/kg of 2-cyclohexene-1-one equivalents.

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

Table 2. Relative odour activity values (rOAV) and odour description by GC-O of volatile compounds in red jujube from different treatments.

Compounds	RAW		FD		B		FR		ST	
	rOAV	GC-O	rOAV	GC-O	rOAV	GC-O	rOAV	GC-O	rOAV	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	oil, fatty; W	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	fatty; W	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; M	143	butter, creamy, sweet; W	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; M	160	sour; W	277	sour; M	126	sour; W
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; W
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	pineapple, fruity; M	45.96	pineapple, fruity; M	67.92	pineapple, fruity; M	1.13	-	8.47	pineapple, fruity; W
Ethyl hexanoate	34.76	fruity; M	44.25	fruity; M	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,; 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	fruity; sweet; M	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	floral; M	<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, jujube; S	2933	sweet, jujube; S	102	sweet, jujube; S	n.d	-	765	sweet, jujube; 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; M	<1	coconut; 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, floral; W	n.d	-	n.d	-	n.d	-	n.d	-
5-Heptyloxolan-2-one	n.d	-	9.9	fruity, sweet; W	7.36	fruity, sweet; W	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.

CRediT authorship contribution statement

Min Gou: Conceptualization, Data curation, Formal analysis, Methodology, Investigation, Writing-original draft & editing. **Jinfeng Bi:** Conceptualization, Resources, Supervision, Writing-review & editing. **Gege Liu:** Investigation. **Marie-Laure Fauconnier**: Supervision, Writing-review & editing. **Qinqin Chen:** Conceptualization, Supervision, Writing-review & editing, Methodology, Validation.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical Abstract