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Comparative nutritional and metabolic analysis reveals the taste variations during yellow rambutan fruit maturation

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ABSTRACT

The metabolic reasons for rambutan taste variations during maturity are unknown. Here, we obtained a unique rambutan cultivar Baoyan No.2 (BY2) with a strong yellow pericarp and excellent taste, the sugar-acid ratios of which ranged from 21.7 to 94.5 during maturation. Widely targeted metabolomics analysis was performed to reveal the metabolic reasons behind these taste variations. The results showed that 51 metabolites were identified as common different metabolites (DMs), including 16 lipids, 12 amino acids and others. Among them, the abundance level of 3,4-digalloylshikimic acid exhibited a positive correlation with the titratable acids (R^2 = 0.9996) and a negative correlation with the sugar-acid ratio ($R^2 = 0.9999$). Therefore, it could be a taste biomarker of BY2 rambutan. Moreover, all DMs were enriched in "galactose metabolism", "fructose and mannose metabolism" and "biosynthesis of amino acids" pathways, which predominantly accounted for the taste variation. Our findings provided new metabolic evidence for the taste variation of rambutan.

1. Introduction

Rambutan (*Nephelium lappaceum* L.) is a subtropical fruit belonging to Sapindaceae spp., the same family as the lychee and longan [\(Wall,](#page-6-0) [2006\)](#page-6-0). Rambutan contains 38.6 %–70.8 % peel, 19.1 %–45.9 % pulp, and 8.3 %–20.3 % seed, which is very popular in Asia ([Kong, Mohd](#page-5-0) [Adzahan, Karim, Rukayadi,](#page-5-0) & Mohd Ghazali, 2018). The distinctive feature of rambutan is its red hair-like protuberance pericarp, and yellow rambutan has never been reported in China. *Nephelium lappaceum* L. cultivar Baoyan No.2 (BY2) is a new popular rambutan cultivar due to its exceptionally yellow appearance and flavor in South China. Rambutan tastes sweet or sour, mainly depending on the growing stage. The pulp, similar to lychee, is a good source of sugars, vitamin C, phenolics, and amino acids [\(Phuong, Le, Van Camp,](#page-5-0) & Raes, 2020; Sunardi & Rozana, [2021\)](#page-5-0). Rambutan is popular among consumers for its well-balanced sweet–sour tastes as well as its antioxidant ([Palanisamy et al., 2008](#page-5-0)), anti-inflammatory ([Li, Li, Hou, Zhuang,](#page-5-0) & Sun, 2018), and anticancer

([Zhang, Zhang,](#page-6-0) & Hao, 2014) effects. The primary metabolites, especially carbohydrates and organic acids, have a close relationship with either the sweet or sour taste of fruits [\(Guo et al., 2015; Ma et al., 2015;](#page-5-0) [Zhu et al., 2020](#page-5-0)). Several researchers have investigated the taste composition of rambutan. It has been reported that rambutan fruit contains a high content of sugars, mainly including 5.38 %–10.01 % sucrose, 1.75 %–3.18 % fructose, and 1.72 %–2.43 % glucose ([Kong](#page-5-0) [et al., 2018\)](#page-5-0). In addition, citric acid is the predominant acid in rambutan pulp ([Lee, Tan, Yu, Curran,](#page-5-0) & Liu, 2013).

Widely targeted metabolomics is a novel detection technology that integrates the "extensiveness" of nontargeted metabolomics with the "accuracy" of targeted metabolomics [\(Feng, Gao, Jiao, Shi,](#page-5-0) & Wang, [2020; Kang et al., 2020\)](#page-5-0). With the self-built compound database and the multiple reaction monitoring (MRM) scanning mode of mass spectrometry, this method can identify hundreds of metabolites qualitatively and quantitatively. Recently, ultra-performance liquid chromatographytandem mass spectrometry (UPLC–MS/MS) based widely targeted

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metabolomics analysis has been successfully used for ultrasensitive detection of a vast number of taste components of subtropic fruits, such as loquat, wampee, and litchi [\(Jiang et al., 2021; Yin, Zhang, Wu, Chen,](#page-5-0) & [Deng, 2022; Yin et al., 2022; Zou et al., 2020](#page-5-0)). Although previous studies have focused on the sugars and organic acids of rambutan, limited data are available detailing the systemic identification of primary and secondary metabolites that govern the unique taste of this fruit. Thus, vital taste-related metabolites and mechanisms during rambutan maturation are still unknown. To date, changes in tasterelated nutrients and metabolites in ripening BY2 rambutan have also never been characterized.

This study aims to reveal the taste metabolic variations between three typical growth stages of the yellow rambutan cultivar BY2. First, the essential nutrients related to taste were compared between growing stages. Then, widely targeted metabolomics analysis was carried out to screen all metabolites in BY2 rambutan. Finally, the characteristic metabolites and potential taste biomarkers were identified, and the possible mechanism was also explained by pathway enrichment analysis. This paper not only sheds new light on the taste-forming mechanism of rambutan but also provides valuable data for rambutan breeding and harvesting.

2. Materials and methods

2.1. Chemicals and reagents

Chromatographic grade fructose, glucose, and sucrose were purchased from the National Institute of Metrology (Beijing, China). Chromatographic grade malic acid, lactic acid, tartaric acid, and citric acid were purchased from Achemtek Co., Ltd. (Worcester, MA, USA). Chromatographic grade acetonitrile, methanol, and formic acid were purchased from Merck (Darmstadt, Germany). All other chemicals were analytical reagent grade.

2.2. Materials and sampling

As shown in Fig. 1, unripe fruit (S1, 20 % maturity, 60 \pm 2 days after flowering, green pericarp, soluble sugar 12.3 %), half-ripe fruit (S2, 50 % maturity, 90 \pm 2 days after flowering, green-yellow pericarp, soluble sugar 14.8 %), and full-ripe fruit (S3, 100 % maturity, 120 ± 2 days after flowering, yellow pericarp, soluble sugar 16.4 %) were obtained from three random trees at the Baoting Tropical Crops Institute (18.6095 N, 109.74039 E). Each growing stage of rambutan had three samples, with at least 45 fruits. Fifteen rambutan pulps were juiced by a blender for two min to determine taste-related qualities and nutrients, including sugars, organic acids, and amino acids.

2.3. Sugar, organic acid, TSS, pH, and amino acid analysis

The content of soluble sugar was measured by the anthrone-sulfuric acid colorimetric method. In brief, 1 mL of 10, 20, 40, 80, and 100 µg/ mL glucose standard solutions in the tubes were added to 4 mL of 0.2 %

anthrone-sulfuric acid solution. After incubating in boiling water for 10 min, these tubes were cooled in water to 25℃. The absorption was measured by a UV–vis spectrophotometer (752 N Plus, Inesa Analytical Instrument Co., Ltd., Shanghai, China). The standard curve was $Y =$ $0.0057x + 0.2541$, $R^2 = 0.9991$. The content of soluble sugar in juiced samples was determined based on the standard curve. The contents of fructose, glucose, and sucrose were determined by the HPLC method according to GB5009.8-2016 (National Food Safety Standards: Determination of Fructose, Glucose, Sucrose, Maltose and Lactose in Foods, CN). The content of titratable acid was determined by 0.02 mol/L sodium hydroxide solution. The contents of malic, citric, lactic, and tartaric acids were determined by the HPLC method according to GB5009.157-2016 (National Food Safety Standards: Determination of Organic Acids in Foods, CN). The content of total soluble solid (TSS) and pH were determined by a Brix refractometer (PAL-1, Atago, Japan), and a pH meter (PH838, Dongguan Wanchuang Electronic Products Co., Ltd., Dongguan, China), respectively. Two grams of juiced samples were put into tubes with 50 mL of pure water and two ceramic protons. Then the tubes were vortexed for 10 min and sonicated in a water bath for 10 min. After being centrifuged at 10,000 r/min for 5 min, the supernatant was filtered (SCAA-104, 0.22 µm; ANPEL, Shanghai, China) before UPLC-MS/MS analysis according to GB/T30987-2020 (Determination of Free Amino Acids in Plant, CN). All samples were measured three times.

2.4. Widely targeted metabolomics analysis

Fifteen rambutan pulps were mixed by a blender for two min. Six hundred microliters of methanol solution (70 %) was added to a 50 mg fruit sample, and the tube vortexed was vortexed three min for extraction. The tube was centrifuged at 12,000 r/min for ten min for separation. The supernatant of the tube was collected after treatment with a 0.22 μm filter.

An UPLC-MS/MS system (UPLC, Nexera X2, Shimadzu; MS, Applied Biosystems 6500 Q TRAP, Japan) was used to identify and quantify all the metabolites. Briefly, a 2 mL filtered sample was loaded onto the UPLC. The mobile phase was solvent A (ultrapure water with 0.1 % formic acid) and solvent B (acetonitrile with 0.1 % formic acid). The mobile phase was programmed as follows: 0–9 min, 5 %–95 % B; 9–10 min, 95 % B; 10–11.1 min, 95 % B-5 % B; 11.1–14 min, 5 % B. Multiple reaction monitoring (MRM) transitions were monitored for effluent metabolites. Five crucial MS parameters, including DP (declustering potential), CE (collision energy), RT (retention time), Q1 (precursor ion), and Q3 (product ion) were used to identify the metabolite from the metware database (Wuhan Metware Biotechnology Co. Ltd). The peak area of the metabolite was used for quantification.

2.5. Identification of differential metabolites

Differential metabolites (DMs) were selected based on two factors. First, metabolites must be differentially accumulated in two growing stages with a fold change (FC) \geq 2, that is, $|\text{Log}_2\text{FC}| \geq 1.0$. Second, a variable importance in projection value (VIP \geq 1) was another factor

Fig. 1. Three growing stages of BY2 rambutan.

used to further screen these metabolites.

2.6. KEGG pathway enrichment analysis of metabolites

Kyoto Encyclopedia of Genes and Genomes (KEGG) is a famous and reliable database for interpreting molecular-level details of genomes, enzymes, and chemicals in organisms [\(Kanehisa, Goto, Sato, Furumichi,](#page-5-0) $&$ [Tanabe, 2012\)](#page-5-0). The above metabolites identified in rambutan were interpreted by the KEGG database. Then, the pathway enrichment analysis was carried out to find the key pathways based on the rich factor and the number of DMs.

2.7. Statistical analysis

SPSS (22.0, IBM Corp., Armonk, NY, USA) software was used for data statistical analysis. OriginLab (2019b, OriginLab Inc, MA, USA) software was used for graphing.

3. Results and discussion

3.1. Sugars and organic acids of BY2 rambutan at different growth stages

Generally, fruit gradually accumulate sugars from unripe to fully ripe (Al-Maiman & [Ahmad, 2002\)](#page-5-0). As shown in Table 1, three typical growing stages, including unripe (S1), half-ripe (S2), and full-ripe (S3) rambutan fruit, were compared. Soluble sugars accumulated from 12.3 % to 16.4 % during ripening. Only sucrose, as the dominant sugar, had the same significant increase as soluble sugar during the three growing stages. In contrast, titratable acid content decreased from unripe to fully ripe. Titratable acid decreased sharply from 0.52 % in S2 to 0.17 % in S3. Four crucial organic acids, including lactic, malic, citric, and tartaric acids, were observed to have the same significant decrease between S2 and S3. The sugar-acid ratio and pH of BY2 rambutan notably increased from 28.6 and 4.1 at S2 to 94.5 and 4.6 at S3, respectively. The above findings demonstrated that the taste of BY2 rambutan improved mainly in the half-ripe to fully ripe stages, and the degradation of organic acids contributed primarily to sugar-acid ratio variations.

Previous studies have confirmed that sugars mainly contribute to the sweet taste of fruit, while the sourness of fruit is often intimately related to organic acids. Both their contents and composition have a complex influence on taste (Chen, Liu, & [Chen, 2009; Ma et al., 2019](#page-5-0)). Fructose, glucose, and sucrose are the vital soluble sugars of most mature fruit, while the composition and concentration of organic acids vary significantly among cultivars and maturities. For example, citric acid is the dominant acid of citrus ([Scherer et al., 2012](#page-5-0)), while malic acid is the primary acid of loquat ([Chen et al., 2009\)](#page-5-0) and apple [\(Ma et al., 2015](#page-5-0)). The malic content increased with maturity, while citric acid decreased during persimmon maturation [\(Senter, Chapman, Forbus,](#page-6-0) & Payne, [1991\)](#page-6-0). We demonstrated that the predominant organic acid of BY2 rambutan was lactic acid, followed by citric, malic, and tartaric acids. Interestingly, the contents of the above four acids showed the same significant decrease between S2 and S3.

3.2. Amino acids of BY2 rambutan at different growth stages

Amino acids, along with sugars and organic acids, are the main metabolites and play a vital role in fruit quality [\(Zhang, Li,](#page-6-0) & Cheng, [2010\)](#page-6-0). In total, 19 amino acids showed noticeable changes during fruit maturation (Table S1). The contents of alanine (Ala), glycine (Gly), and γ-amino butyric acid (GABA) increased throughout fruit ripening. In contrast, aspartic acid (Asp) decreased gradually from 69.8 mg/kg in S1 to 35.4 mg/kg in S3. Another 15 amino acids, including arginine (Arg), asparagine (Asn), glutamine (Gln), glutamic acid (Glu), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), and valine (Val), showed a significant decrease from half-ripe to fullripe. In mature BY2 rambutan fruit, Ala, GABA, Gln, and Lys were the primary amino acids, mainly contributing to the flavor. Leu and Ile remained relatively stable until the fruit was fully mature.

In summary, the contents of most sugars remained relatively unchanged from the half-ripe to full-ripe stage of BY2 rambutan. In contrast, all organic acids along with the majority of amino acids decreased sharply from stage S2 to S3, suggesting that most sugars accumulated at the same rate with fruit growth, whereas both organic acids and amino acids were synthesized at a slower rate with fruit maturation. Until now, no data were found on the comparative analyses of all metabolites, especially components related to sugars, acids, and amino acids in different growing stages of rambutan. Thus, widely targeted metabolomics analysis was carried out to investigate the tasterelated component variations and underlying mechanism during maturation.

3.3. Widely targeted metabolomics analysis

With the advancement of chromatography technology, widely targeted metabolomics analysis has been extensively applied to identify numerous metabolites in fruits and vegetables [\(Jiang et al., 2021; Yang](#page-5-0) [et al., 2021; Zhang et al., 2020](#page-5-0)). There were 821 metabolites identified in all (Table S2), including sugars, organic acids, amino acids, flavones, and others. Principal component analysis (PCA) was applied to uncover the connection of all groups. [Fig. 2A](#page-3-0) showed that the contributions of PC1, PC2, and PC3 were 34.67 %, 24.03 %, and, 13.07 % respectively. The total contribution of PC1, PC2, and PC3 was 71.77 %, which covered the major information of 821 metabolites. Four groups, including BY2- S1, BY2-S2, BY2-S3, and quality control (QC), were clearly separated, and the three samples within the groups were closely clustered together. Hierarchical cluster analysis (HCA) also demonstrated that all chemical classes, especially flavonoids, lipids, and phenolic acids, accumulated differently among the three growing stages ([Fig. 2B](#page-3-0)). The above PCA and HCA analyses both proved that the BY2-S1, BY2-S2, and BY2-S3 rambutans were three distinct groups with individual metabolite profiles.

3.4. Identification and classification of DMs

To identify DMs in rambutan of three growing stages, we selected

Table 1 Sugars, acids, and basic taste qualities of BY2 rambutan at three growing stages.

Note: Sugar-acid ratio is soluble sugar divided by titratable acid. Different letters on the number meant significant differences between growing stages (*p <* 0.05).

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Fig. 2. PCA and HCA analysis of BY2 rambutan. (A) Principal component analysis of metabolites identified from BY2 rambutan at three growing stages. Each growing stage had three individual samples. For example, BY2-S1-1, BY2-S1-2, and BY2-S1-3 were three BY2-S1 rambutan samples. Equal volumes of BY2-S1, BY2- S2, and BY2-S3 flesh samples were mixed for use as quality control (OC). (B) Hierarchical cluster analysis of metabolites identified from BY2 rambutan. The color from green (low) to red (high) indicates the level of each metabolite. The Z-score represents the deviation from the mean by standard deviation units. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

metabolites based on $|Log_2FC| \ge 1.0$ and VIP ≥ 1 . In total, 402 DMs were identified, which could be divided into 13 classes, and major DMs were flavonoids, amino acids and derivatives, and phenolic acids (Fig. 3B). There were 232, 205, and 275 DMs in BY2-S1 vs BY2-S2, BY2-S2 vs BY2- S3, and BY2-S1 vs BY2-S3, respectively. Recent studies also found that rambutan contains high contents of flavonoids and phenolic acids in either pulp or pericarp [\(Chaiwarit et al., 2021; Li, Yu,](#page-5-0) & Xu, 2017; [Phuong et al., 2020](#page-5-0)). We focused on the main taste metabolites, including sugars, organic acids, and amino acids. Then Venn diagram analysis was applied.

Fifty-one differentially accumulated metabolites in three growing stages were identified as common DMs in BY2 rambutan (Fig. 3A). There were 16 lipids, 12 amino acids and derivatives, 9 flavonoids, 4 phenolic acids, and 10 other metabolites (Table S3). A considerable number of lipids and flavonoids showed apparent differences between growing stages, which could partly explain the color difference of rambutan fresh shown in [Fig. 1.](#page-1-0) What is interesting about the data in Table S3 is that 12 amino acids and derivatives were found accumulate differently in all stages from S1 to S3, indicating that amino acids were actively synthesized or decomposed during maturation. This is consistent with the findings in Table S1. Notably, four phenolic acids, including 2,6-dimethoxy-hydroquinone-4-*O*-β-D-glucopyranoside, vanillic acid, 3-*O*-p-coumaroylshikimic acid, and 3,4-digalloylshikimic acid varied differently during maturation. Among all phenolic acids, only 3,4-digalloylshikimic acid was observed to decrease continuously with increasing maturity of BY2 rambutan.

3.5. KEGG enrichment analysis of DMs

The DMs in the three growing stages of BY2 rambutan were interpreted by the KEGG database, and the main metabolic pathways were shown in Fig. S1. There were 20 metabolic pathways, but mainly sugar metabolism and amino acid biosynthesis, including "galactose metabolism", "fructose and mannose metabolism", "biosynthesis of amino

Fig. 3. Venn diagram (A) and classification (B) of differential metabolites in BY2 rambutan.

acids", "valine, leucine, and isoleucine biosynthesis", "arginine and proline biosynthesis", and "arginine biosynthesis" pathways. According to the rich factor, the DMs were primarily enriched in the "valine, leucine, and isoleucine biosynthesis" pathway, followed by the "flavone and flavonol biosynthesis" and the "fructose and mannose metabolism" pathways. There were 21 DMs found in the "biosynthesis of amino acids" pathway, 8 DMs in the "arginine and proline biosynthesis" pathway, and 5 DMs in the "arginine biosynthesis" pathway. This proved that massive amounts of metabolites participated in the synthesis of amino acids, which greatly improved the nutritional value and taste of rambutan during maturation.

As shown in Fig. 4, the map provided comprehensive insight into the mechanism underlying the taste variations based on the five main KEGG pathways. Three dominant sugars (sucrose, fructose, glucose) accumulated from the S2 to S3 stages due to active "galactose metabolism" and "fructose and mannose metabolism" pathways. Sucrose was observed to be relatively stable in either the S2 or S3 growing stage because of its continuous transformation to fructose and glucose in rambutan fruit. The content of lactose was also found to be stable from the unripe to fully ripe fruit of rambutan, demonstrating that the synthesis or decomposition of lactose was active and well-balanced during maturation. Almost all amino acids were observed to accumulate differently during fruit maturation. Except for Ile, 17 other amino acids were all decreased or unchanged from the unripe to fully ripe fruit of BY2 rambutan. Pyruvate entered the "biosynthesis amino acids" pathway through 2-oxoglutarate, leading to an obvious drop in Glu and glutamine. Glu, a precursor of Arg, is actively engaged in amino acid metabolism by assimilating and dissimilating ammonia (Forde $&$ [Lea, 2007](#page-5-0)). Therefore, Arg decreased obviously with Glu during the S2 and S3 growth stages of BY2 rambutan. Our widely targeted metabolomics analysis also detected lipids, lignans, coumarins, alkaloids, and other metabolites. However, few DMs were found in five main KEGG pathways. Thus, they seem to contribute little to the taste variation among the three typical growing stages of BY2 rambutan.

3.6. Potential taste biomarker

Among all 51 common DMs, only 3,4-digalloylshikimic acid decreased continuously with increasing maturity and the sugar-acid ratio of BY2 rambutan. We speculated that it could be a potential taste biomarker of BY2 rambutan. Before correlation analysis, the peak area of 3,4-digalloylshikimic acid was log_{10} transformed. As shown in Fig. 5, the abundance level of 3,4-digalloylshikimic acid showed a positive correlation with the titratable acids ($R^2 = 0.9996$) and a negative correlation with the sugar-acid ratio ($R^2 = 0.9999$) in S1, S2, and S3 rambutan. Thus, it could be a taste biomarker of BY2 rambutan.

3,4-Digalloylshikimic acid is a derivative of shikimic acid, which is an essential precursor for several aromatic amino acids and other compounds such as alkaloids, phenolics, and phenylpropanoids in plants (Johansson & [Liden, 2006; Treadway et al., 2009\)](#page-5-0). That is why three

Fig. 5. Correlation between 3,4-digalloylshikimic acid, sugar acid ratio and titratable acids in BY2 rambutan.

Fig. 4. KEGG map of key DMs in BY2 rambutan. This map is constructed based on the KEGG pathway and literary references. Colored boxes in front of each metabolite indicate \log_{10} S1, \log_{10} S2, and \log_{10} S3 values according to the color scale. * represents the taste biomarker.

aromatic amino acids, including Tyr, Try, and Phe, were found to sharply decrease with the decrease of 3,4-digalloylshikimic acid from the S2 to S3 growing stages. Tyr, Try, and Phe are central amino acids of plants and essential amino acids of humans. Both of them cannot be synthesized in the body (Galili & Hofgen, 2002). Importantly, Try, along with Lys and Met, greatly improves the nutritional quality of plant foods (Wakasa $&$ [Ishihara, 2009](#page-6-0)). In addition, p-fructose and fructose-6P were the precursors of shikimic acid as well as 3,4-digalloylshikimic acid. We proved that the gradual accumulation of fructose in BY2 rambutan during maturation was actively regulated by a decrease of 3,4-digalloylshikimic acid. Therefore, the sugar-acid ratio as well as amino acids showed significant variations from half-ripe to full-ripe, and the taste improved gradually.

4. Conclusions

In this paper, we applied taste-related quality analysis combined with widely targeted metabolomics analysis to BY2 rambutan, which is a special cultivar with a strong yellow appearance and exquisite taste depending on maturation. The taste of BY2 rambutan was greatly improved from S2 to S3, with dominant sugars insignificantly changing, while four main organic acids and 15 amino acids decreased sharply. There were 232, 205, and 275 metabolites differed in S1 vs S2, S2 vs S3, and S1 vs S3, respectively. Fifty-one metabolites were identified as DMs, including 16 lipids, 12 amino acids and derivatives, 9 flavonoids, 4 phenolic acids, and 10 other metabolites. Among all phenolic acids, 3,4 digalloylshikimic acid could be a potential taste biomarker, showing a positive correlation with titratable acids ($R^2 = 0.9996$) and a negative correlation with the sugar-acid ratio($R^2 = 0.9999$) in S1, S2, and S3 rambutan. Moreover, all DMs were enriched in "galactose metabolism", "fructose and mannose metabolism" and "biosynthesis of amino acids" pathways, which predominantly accounted for taste variation during rambutan maturation.

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CRediT authorship contribution statement

Hao Deng: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. **Guang Wu:** Investigation, Data curation, Writing – review & editing. **Ronghu Zhang:** Formal analysis, Writing – review & editing. **Qingchun Yin:** Formal analysis, Data curation. **Bin Xu:** Formal analysis, Investigation. **Liying Zhou:** Visualization. **Zhe Chen:** Supervision, Funding acquisition, Writing – review $&$ editing.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

Acknowledgment

Quantification of metabolites is done using the database of Wuhan Metware Biotechnology Co. Ltd (Wuhan, China).

Appendix A. Supplementary data

Supplementary data (Figure S1. Key KEGG pathways enrichment; Table S1. Amino acids of BY2 rambutan at three growing stages (mg/ kg); Table S2. 821 metabolites in BY2 rambutan; Table S3. 51 common differential metabolites in BY2 rambutan) to this article can be found online at<https://doi.org/10.1016/j.fochx.2023.100580>.

References

- Al-Maiman, S. A., & Ahmad, D. (2002). Changes in physical and chemical properties during pomegranate (Punica granatum L.) fruit maturation. *Food Chemistry, 76*(4), 437-441. https://doi.org/10.1016/s0308-8146(01)00301-6.
- Chaiwarit, T., Kantrong, N., Sommano, S. R., Rachtanapun, P., Junmahasathien, T., Kumpugdee-Vollrath, M., & Jantrawut, P. (2021). Extraction of tropical fruit peels and development of HPMC film containing the extracts as an active antibacterial packaging material. *Molecules, 26*(8). https://doi.org/10.3390/molecules26082265
- Chen, F., Liu, X., & Chen, L. (2009). Developmental changes in pulp organic acid concentration and activities of acid-metabolising enzymes during the fruit development of two loquat (Eriobotrya japonica Lindl.) cultivars differing in fruit acidity. *Food Chemistry, 114*(2), 657–664. [https://doi.org/10.1016/j.](https://doi.org/10.1016/j.foodchem.2008.10.003) [foodchem.2008.10.003](https://doi.org/10.1016/j.foodchem.2008.10.003)
- Feng, Z. H., Gao, Z. F., Jiao, X., Shi, J. F., & Wang, R. F. (2020). Widely targeted metabolomic analysis of active compounds at different maturity stages of 'Hupingzao' jujube. *Journal of Food Composition and Analysis, 88*, Article 103417. //doi.org/10.1016/j.jfca.2020.103417
- Forde, B. G., & Lea, P. J. (2007). Glutamate in plants: Metabolism, regulation, and signalling. *Journal of Experimental Botany, 58*(9), 2339–2358. [https://doi.org/](https://doi.org/10.1093/jxb/erm121) [10.1093/jxb/erm121](https://doi.org/10.1093/jxb/erm121)
- Galili, G., & Hofgen, R. (2002). Metabolic engineering of amino acids and storage proteins in plants. *Metabolic Engineering, 4*(1), 3–11. [https://doi.org/10.1006/](https://doi.org/10.1006/mben.2001.0203) [mben.2001.0203](https://doi.org/10.1006/mben.2001.0203)
- Guo, S., Duan, J. A., Qian, D., Tang, Y. P., Wu, D. W., Su, S. L., … Zhao, Y. (2015). Content variations of triterpenic acid, nucleoside, nucleobase, and sugar in jujube (Ziziphus jujuba) fruit during ripening. *Food Chemistry, 167*, 468–474. [https://doi.](https://doi.org/10.1016/j.foodchem.2014.07.013) [org/10.1016/j.foodchem.2014.07.013](https://doi.org/10.1016/j.foodchem.2014.07.013)
- Jiang, N., Zhu, H., Liu, W., Fan, C., Jin, F., & Xiang, X. (2021). Metabolite Differences of polyphenols in different Litchi cultivars (Litchi chinensis Sonn.) based on extensive targeted metabonomics. *Molecules, 26*(4). [https://doi.org/10.3390/](https://doi.org/10.3390/molecules26041181) [molecules26041181](https://doi.org/10.3390/molecules26041181)
- Johansson, L., & Liden, G. (2006). Transcriptome analysis of a shikimic acid producing strain of Escherichia coli W3110 grown under carbon- and phosphate-limited conditions. *Journal of Biotechnology, 126*(4), 528–545. [https://doi.org/10.1016/j.](https://doi.org/10.1016/j.jbiotec.2006.05.007) [jbiotec.2006.05.007](https://doi.org/10.1016/j.jbiotec.2006.05.007)
- Kanehisa, M., Goto, S., Sato, Y., Furumichi, M., & Tanabe, M. (2012). KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Research, 40*, 109–114. <https://doi.org/10.1093/nar/gkr988>
- Kang, C., Lv, C., Yang, J., Kang, L., Ma, W., Zhang, W., … Guo, L. (2020). A practical protocol for a comprehensive evaluation of sulfur fumigation of trichosanthis radix based on both non-targeted and widely targeted metabolomics. *Frontier in Plant Science., 11*, Article 578086. <https://doi.org/10.3389/fpls.2020.578086>
- Kong, F. C., Mohd Adzahan, N., Karim, R., Rukayadi, Y., & Mohd Ghazali, H. (2018). Selected physicochemical properties of registered clones and wild types rambutan (Nephelium lappaceum L.) fruits and their potentials in food products. *Sains Malaysiana, 47*(7), 1483–1490. <https://doi.org/10.17576/jsm-2018-4707-16>
- Lee, P. R., Tan, R. M., Yu, B., Curran, P., & Liu, S. Q. (2013). Sugars, organic acids, and phenolic acids of exotic seasonable tropical fruits. *Nutrition & Food Science, 43*(3), 267–276. <https://doi.org/10.1108/00346651311327927>
- Li, P., Yu, X., & Xu, B. (2017). Effects of UV-C Light exposure and refrigeration on phenolic and antioxidant profiles of subtropical fruits (litchi, longan, and rambutan) in different fruit forms. *Journal of Food Quality, 2017*, 1–12. [https://doi.org/](https://doi.org/10.1155/2017/8785121) [10.1155/2017/8785121](https://doi.org/10.1155/2017/8785121)
- Li, Y., Li, Z., Hou, H., Zhuang, Y., & Sun, L. (2018). Metal chelating, inhibitory DNA amage, and anti-inflammatory activities of phenolics from rambutan (Nephelium lappaceum) peel and the quantifications of geraniin and corilagin. *Molecules, 23*(9). <https://doi.org/10.3390/molecules23092263>
- Ma, B., Chen, J., Zheng, H., Fang, T., Ogutu, C., Li, S., … Wu, B. (2015). Comparative assessment of sugar and malic acid composition in cultivated and wild apples. *Food Chemsitry, 172*, 86–91. <https://doi.org/10.1016/j.foodchem.2014.09.032>
- Ma, B., Ding, Y., Li, C., Li, M., Ma, F., & Yuan, Y. (2019). Comparative proteomic analysis reveals key proteins linked to the accumulation of soluble sugars and organic acids in the mature fruits of the wild malus species. *Plants (Basel), 8*(11). [https://doi.org/](https://doi.org/10.3390/plants8110488) [10.3390/plants8110488](https://doi.org/10.3390/plants8110488)
- Palanisamy, U., Cheng, H. M., Masilamani, T., Subramaniam, T., Ling, L. T., & Radhakrishnan, A. K. (2008). Rind of the rambutan, Nephelium lappaceum, a potential source of natural antioxidants. *Food Chemistry, 109*(1), 54–63. [https://doi.](https://doi.org/10.1016/j.foodchem.2007.12.018) [org/10.1016/j.foodchem.2007.12.018](https://doi.org/10.1016/j.foodchem.2007.12.018)
- Phuong, N. N. M., Le, T. T., Van Camp, J., & Raes, K. (2020). Evaluation of antimicrobial activity of rambutan (Nephelium lappaceum L.) peel extracts. *International Journal of Food Microbiology, 321*, Article 108539. [https://doi.org/10.1016/j.](https://doi.org/10.1016/j.ijfoodmicro.2020.108539) ijfoodmicro.2020.10853
- Scherer, R., Rybka, A. C. P., Ballus, C. A., Meinhart, A. D., Filho, J. T., & Godoy, H. T. (2012). Validation of a HPLC method for simultaneous determination of main

organic acids in fruits and juices. *Food Chemistry, 135*(1), 150–154. [https://doi.org/](https://doi.org/10.1016/j.foodchem.2012.03.111) [10.1016/j.foodchem.2012.03.111](https://doi.org/10.1016/j.foodchem.2012.03.111)

- Senter, S. D., Chapman, G. W., Forbus, W. R., & Payne, J. A. (1991). Sugar and nonvolatile acid composition of persimmons during maturation. *Journal of Food Science, 56*(4), 989-991. https://doi.org/10.1111/j.1365-2621.1991.tb14623.
- Sunardi, S., & Rozana, R. (2021). Minimally process pada buah rambutan dan perubahan kandungan vitamin C selama penyimpanan beku. *Journal of Food Technology and Agroindustry, 3*(1), 36–44. <https://doi.org/10.24929/jfta.v3i1.1247>
- [Treadway, M. T., Grant, M. M., Ding, Z., Hollon, S. D., Gore, J. C., & Shelton, R. C.](http://refhub.elsevier.com/S2590-1575(23)00022-6/h0115) [\(2009\). Early adverse events, HPA activity and rostral anterior cingulate volume in](http://refhub.elsevier.com/S2590-1575(23)00022-6/h0115) MDD. *[PLoS One, 4](http://refhub.elsevier.com/S2590-1575(23)00022-6/h0115)*(3), e4887.
- Wakasa, K., & Ishihara, A. (2009). Metabolic engineering of the tryptophan and phenylalanine biosynthetic pathways in rice. *Plant Biotechnology, 26*(5), 523–533. <https://doi.org/10.5511/plantbiotechnology.26.523>
- Wall, M. M. (2006). Ascorbic acid and mineral composition of longan (Dimocarpus longan), lychee (Litchi chinensis) and rambutan (Nephelium lappaceum) cultivars grown in Hawaii. *Journal of Food Composition and Analysis, 19*(6–7), 655–663. <https://doi.org/10.1016/j.jfca.2005.12.001>
- Yang, R., Li, Y., Zhang, Y., Huang, J., Liu, J., Lin, Z., … Wang, B. (2021). Widely targeted metabolomics analysis reveals key quality-related metabolites in kernels of sweet corn. *International Journal of Genomics, 2021*, 2654546. [https://doi.org/10.1155/](https://doi.org/10.1155/2021/2654546) [2021/2654546](https://doi.org/10.1155/2021/2654546)
- Yin, Q., Zhang, R., Wu, G., Chen, Z., & Deng, H. (2022). Comparative metabolomics analysis reveals the taste variations among three selected wampee cultivars. *Plant*

Foods for Humam Nutrition, 77(2), 250–257. [https://doi.org/10.1007/s11130-022-](https://doi.org/10.1007/s11130-022-00973-4) 0097

- Yin, Q. C., Ji, J. B., Zhang, R. H., Duan, Z. W., Xie, H., Chen, Z., … Deng, H. (2022). Identification and verification of key taste components in wampee using widely targeted metabolomics. *Food Chemistry: X, 13*, Article 100261. https://doi.org/ [10.1016/j.fochx.2022.100261](https://doi.org/10.1016/j.fochx.2022.100261)
- Zhang, J., Zhang, W., & Hao, Y. (2014). Multipulse chaotic dynamics for nonautonomous nonlinear systems and application to a FGM plate. *International Journal of Bifurcation and Chaos, 24*(5). https://doi.org/ 10.1142/s0218127414500680.
- Zhang, X., Li, X., Su, M., Du, J., Zhou, H., Li, X., & Ye, Z. (2020). A comparative UPLC-Q-TOF/MS-based metabolomics approach for distinguishing peach (Prunus persica (L.) Batsch) fruit cultivars with varying antioxidant activity. *Food Research International, 137*, 109531. https://doi.org/10.1016/j.foodres.2020.109531.
- Zhang, Y., Li, P., & Cheng, L. (2010). Developmental changes of carbohydrates, organic acids, amino acids, and phenolic compounds in 'Honeycrisp' apple flesh. *Food Chemistry, 123*(4), 1013-1018. https://doi.org/ 10.1016/j.foodchem.2010.05.053.
- Zhu, T., Zuo, W., Yan, J., Wen, P., Pei, Z., Lian, H., & Yang, H. C. (2020). Comparative assessment of the antioxidant activities among the extracts of different parts of clausena lansium (Lour.) skeels in human gingival fibroblast cells. *Evidence-Based Complementary and Alternative Medicine, 2020*, 3958098. https://doi.org/ 10.1155/ 2020/3958098.
- Zou, S., Wu, J., Shahid, M. Q., He, Y., Lin, S., Liu, Z., & Yang, X. (2020). Identification of key taste components in loquat using widely targeted metabolomics. *Food Chemistry, 323*, 126822. https://doi.org/ 10.1016/j.foodchem.2020.126822.