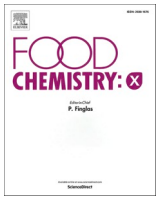
Food Chemistry: X 23 (2024) 101528

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Biomarkers of the main nutritional components in purple rice during five successive grain filling stages

Qiangqiang Xiong [a,b,c,1], Yanyao Lu [a,1], Wenfei Gu [a,1], Yu Zhang [a], Ao Li [d], Shuo Cai [c,*], Nianbing Zhou [a,b,**]

[a] Jiangsu Key Laboratory of Crop Genetics and Physiology/Jiangsu Key Laboratory of Crop Cultivation and Physiology, Agricultural College of Yangzhou University, Yangzhou 225009, China
[b] Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, China
[c] Jiangxi Irrigation Experiment Central Station, Nanchang 330201, China
[d] Shanghai Agrobiological Gene Center, Shanghai 201106, China

ARTICLE INFO

Keywords:
Purple rice
Grain filling
Amino acids
Nutritional components
Mineral elements

ABSTRACT

Differences in main nutritional components in relation to biomarkers of metabolites in purple rice grains at different fillings stages have not been determined previously. This study measured the contents of amino acids, several nutritional indicators, and mineral elements in purple rice grains at five stages following the filling stage. The results revealed that the amino acid, ascorbic acid, total sugar, carotenoid, vitamin B9, cyanidin-3-O-glucoside, peonidin 3-glucoside and seven minerals were highest in the final stage of grain filling. Citric acid, L-isoleucine, trigonelline, and L-glutamate are key metabolites in the metabolic pathway and exhibit strong correlations with various nutritional indicators. Hence, this research preliminarily suggested that trigonelline, L-isoleucine, L-glutamate, and citric acid could be potential biomarkers of nutritional components in purple rice grains during various postfilling stages.

1. Introduction

Rice, one of the primary staple foods worldwide, is highly important in everyday diets. China, as a leading producer and exporter of rice globally, emphasizes the considerable research value of enhancing the nutritional content of rice for the improvement of human health (Sangma & Parameshwari, 2023). China boasts a rich abundance of rice germplasm resources with a wide array of varieties. There are substantial differences in nutritional components among different rice varieties (Zhou et al., 2022). With the advancements in science and technology, people have largely addressed issues of food security, leading to a heightened focus on the nutritional aspects of food. Enhancing the nutritional quality of food can effectively improve human nutrition and health (Mohidem, Hashim, Shamsudin, & Che Man, 2022). In recent years, with the continuous improvement and replacement of rice varieties, rice with high nutritional value has gained increasing favour in the market. Purple (black) rice, which is considered of high nutritional value, has garnered considerable interest (Gu et al., 2023; Pushpa, Sassikumar, Suresh, & Iyanar, 2022). The pigments in purple (black) rice plants possess strong antioxidant activity and the ability to scavenge free

Abbreviations: TICs, total ion current chromatograms; XICs, extracted ion chromatograms; RSD, relative standard deviation; 4-Hpro, 4-hydroxyproline; b-Ala, beta-alanine; GABA, gamma-aminobutyric acid; Ala, L-alanine; Arg, L-arginine; Asn, L-asparagine; Asp, L-aspartic acid; Cys, L-cysteine; Glu, L-glutamic acid; Gln, L-glutamine; Gly, L-glycine; His, L-histidine; Ile, L-isoleucine; Leu, L-leucine; Lys, L-lysine; Met, L-methionine; Orn, L-ornithine; Phe, *L*-phenylalanine; Pro, L-proline; Ser, L-serine; Thr, L-threonine; Trp, L-tryptophan; Tyr, L-tyrosine; Val, L-valine; Tau, taurine; AsA, ascorbic acid; TS, total sugar; CAROT, carotenoid; VB9, vitamin B9; CF, Crude fat; CP, crude protein; C3OG, cyanidin-3-O-glucoside; P3G, peonidin 3-glucoside; ATP, adenosine triphosphate; Fe, iron; Mn, manganese; Zn, Zinc; Cu, Copper; Ca, Calcium; Mg, Magnesium; Se, selenium; ROC, receiver operating characteristic, After 7 days (D7), 14 days (D14), 21 days (D21), 28 days (D28), and 35 days (D35) following the filling of the rice grains..

* Corresponding author.
** Corresponding author at: Jiangsu Key Laboratory of Crop Genetics and Physiology/Jiangsu Key Laboratory of Crop Cultivation and Physiology, Agricultural College of Yangzhou University, Yangzhou 225009, China.
E-mail addresses: caishuo0911@163.com (S. Cai), 008007@yzu.edu.cn (N. Zhou).
[1] Qiangqiang Xiong, Yanyao Lu and Wenfei Gu contributed equally to this manuscript.

https://doi.org/10.1016/j.fochx.2024.101528
Received 3 February 2024; Received in revised form 30 May 2024; Accepted 31 May 2024
Available online 3 June 2024

radicals (Tai, Huang, Zhao, & Huang, 2021). Their structural foundation lies in the copigmentation system formed by a 3-aromatic ring structure. In addition, melanin also plays a role in lowering cholesterol levels, which helps prevent and treat cardiovascular diseases (Kowsalya, Sharanyakanth, & Mahendran, 2022; Sangma & Parameshwari, 2023). Research indicates that, compared to regular white rice, purple (black) rice contains higher levels of amino acids, mineral elements, vitamins, and other substances, suggesting that it has greater dietary and health-promoting value (Wisetkomolmat et al., 2022; Zhang et al., 2022). In previous research, data on the metabolomic and ionomic characteristics of 63 different types of coloured rice were obtained, revealing the presence of 625 metabolites and 22 metal ions. Additional examination revealed comparatively elevated buildup of fragrant compounds and fats in black rice, whereas brown rice exhibited increased accumulation of trace elements such as zinc, copper, and selenium ions (Sedeek et al., 2023). The nutritional composition of purple (black) rice varies throughout the different growth stages. Understanding the nutritional changes of purple rice at five successive grain filling stages is crucial for ensuring the detection of nutrient related biomarkers and promoting the creation of high nutrient rice.

Amino acids serve as the building blocks of life and form the primary framework of proteins. Increasing the amino acid levels in rice could be a potential dietary supplement for communities that depend on rice as a primary source of sustenance (Huang, Liao, Xie, Chen, & Cao, 2023). Researchers have identified the crucial gene *OsAUX5*, which regulates the gathering of vital amino acids in rice grains. This finding illuminates the molecular process through which *OsWRKY78-OsAUX5* controls the buildup of crucial amino acids in rice grains, potentially providing opportunities for developing rice cultivars with elevated levels of essential amino acids (Shi et al., 2023). Rice lipids predominantly consist of triglycerides, phospholipids, and free fatty acids and are distributed within the bran, embryo, and endosperm of the rice grain. Previous studies have identified 99 quantitative train loci associated with 11 lipid-related traits in rice. Among these genes, four genes that contribute significantly to natural variations in lipid composition have been cloned. Additionally, a potential pathway for rice grain lipid biosynthesis has been proposed (Zhou et al., 2021). Vitamins are a collective term for organic substances necessary for maintaining normal physiological functions in the human body and must be obtained from daily dietary intake. Vitamins are vital for physiological functions, the production of necessary nutrients, and metabolic processes. They also aid in preventing anaemia and enhancing immune responses in humans (Bedhiafi et al., 2022). Purple rice grains contain Fe, which can ameliorate nutritional anaemia, while Zn can enhance immunity. The flavonoids present in purple rice can lower blood pressure, and dietary fibre can facilitate intestinal motility, aiding in the prevention and management of obesity, cardiovascular diseases, and constipation (Bedhiafi et al., 2022; Panda, Rani, Behera, Pradhan, & Lenka, 2023). Therefore, purple rice is a successful material for accomplishing the concept of “food as medicine”.

With the continuous improvement of high-throughput metabolite detection techniques and databases, annotating differentially abundant metabolites into relevant metabolic pathways enables the systematic elucidation of the metabolic foundation underlying specific biological functions (Xiong et al., 2022). The aim of the present study measured the contents of amino acids, major nutritional components, and seven mineral elements in purple rice grains at five successive grain filling stages. Furthermore to evaluate the correlation among amino acids, nutrients, and minerals with biomarkers of metabolites, in order to improving the nutritional value of rice, which will establish a theoretical basis for enhancing the high-quality nutritional characteristics of rice.

2. Materials and methods

2.1. Rice planting and environmental conditions

Yangzino 1, which was cultivated at the Shatou Base in Guangling District, Yangzhou city, Jiangsu Province (Zhu et al., 2022), served as the experimental material. The rice was sown on May 20, 2022, and the plants were subsequently cultivated using the mat method for 25 days. Four rice plants were transplanted per hill. The cultivation employed equal row spacings, with a distance of 12 cm between plants and 28 cm between rows, and protective rows were placed on both sides. Rice cultivation was performed in triplicate. Rice fertilization involved the application of compound fertilizer, which contained equal proportions of nitrogen, phosphorus, and potassium at a ratio of 15%. Nitrogen fertilizer was applied at a rate of 300 kg per hectare of pure nitrogen.

2.2. Sample collection

At the heading stage, uniformly sized panicles were selected across all plots and marked with small tags. The marked panicles were collected and quickly frozen using liquid nitrogen after 7 days (D7), 14 days (D14), 21 days (D21), 28 days (D28), and 35 days (D35) following the filling of the rice grains. The grain samples for each growth stage are repeated three times.

2.3. Preparation of the amino acid standard solution

The grain samples for each growth stage are repeated three times. Specifically, 0.03 g of each amino acid standard compound was measured, which included 4-hydroxyproline (4-Hpro), beta-alanine (b-Ala), gamma-aminobutyric acid (GABA), L-alanine (Ala), L-arginine (Arg), L-asparagine (Asn), L-aspartic acid (Asp), L-cysteine (Cys), L-glutamic acid (Glu), L-glutamine (Gln), L-glycine (Gly), L-histidine (His), L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys), L-methionine (Met), L-ornithine (Orn), *L*-phenylalanine (Phe), L-proline (Pro), L-serine (Ser), L-threonine (Thr), L-tryptophan (Trp), L-tyrosine (Tyr), L-valine (Val), and taurine (Tau). To prepare a standard stock solution with a concentration of 1 mg mL^{-1} for each substance, these compounds should be dissolved in water. A mixed standard solution was prepared using a gradient-diluted stock solution with concentrations of 64,000 ng mL^{-1}, 32,000 ng mL^{-1}, 16,000 ng mL^{-1}, 8000 ng mL^{-1}, 4000 ng mL^{-1}, 2000 ng mL^{-1}, 1000 ng mL^{-1}, 500 ng mL^{-1}, 250 ng mL^{-1}, 125 ng mL^{-1}, 62.5 ng mL^{-1}, and 31.25 ng mL^{-1}. The content of each amino acid was determined following the method described in Xiong et al. (2022).

2.4. Determination of several nutritional components

The grain samples for each growth stage are repeated three times. Crude fat (CF) concentrations were measured in accordance with the National Standards of the People's Republic of China (NSPRC, 2017a). The crude protein (CP) content was determined following the NSPRC (NSPRC, 2017b) guidelines. Suzhou Michy Biomedical Technology Co., Ltd., located in Suzhou, China, determined the ascorbic acid (AsA) and carotenoid (CAROT) contents (Sarker & Oba, 2021), as well as the total sugar (TS) content (NSPRC, 2009). The concentration of plant elements was analysed using inductively coupled plasma–mass spectrometry/atomic emission spectrometry (ICP–MS/AES) (Thermo Fisher Scientific, Waltham, MA, USA) following the NSPRC guidelines (NSPRC, 2017c). The adenosine triphosphate (ATP) content was determined according to the method outlined in Xiong et al. (2023). To determine the content of cyanidin-3-O-glucoside (C3OG) and peonidin 3-glucoside (P3G), consult the procedure outlined in Zhu et al. (2022). The ATP standard curve, which encompassed the linear range and correlation coefficients, was determined (Table S1).

2.5. Determination of vitamin B9 (VB9) concentration

The grain samples for each growth stage are repeated three times. A total of 0.2 g of sample was weighed, and 1.0 mL of 0.5% ammonia solution was quickly added under low-light and low-temperature conditions. The mixture was ground into a slurry using a grinder, ultrasonic

extraction was performed for 20 min, after which the mixture was centrifuged, and the supernatant was collected. The volume was adjusted to 1.0 mL, the sample was filtered using a needle-type filter, and the solution was ready for testing. The HPLC analysis conditions for VB9 were as follows: the chromatograph used was an Agilent 1100 HPLC system with a UV detector set at a wavelength of 254 nm. The chromatographic column used was a Compass C18 (2) reversed-phase column with dimensions of 250*4.6 mm and a particle size of 5 μm. The column temperature was maintained at 30 °C, and the flow rate was set at 1.0 mL min^{-1}. The injection volume was 10 μL. Mobile phase A consisted of 0.1% phosphoric acid in water, while mobile phase B was methanol. The ratio of mobile phase A to mobile phase B was 70:30.

To determine the standard curve, the VB9 standard substance was accurately measured, the mixture was dissolved in a solution containing 0.5% ammonia, and standard solutions with mass concentrations of 0.04 μg mL^{-1}, 0.1 μg mL^{-1}, 0.4 μg mL^{-1}, 1 μg mL^{-1}, 4 μg mL^{-1}, 10 μg mL^{-1}, and 40 μg mL^{-1} were created. Following the aforementioned chromatographic conditions, the peak areas of each standard solution were sequentially analysed. The standard curve for VB9 was generated by plotting the concentration on the horizontal axis and the peak area on the vertical axis, which also included the linear range and correlation coefficients (Table S2).

2.6. Physiological and biochemical data analysis

All samples were repeated three times for statistical purposes. The physiological and biochemical data of the seeds were organized, mean values were calculated, and graphs were generated using WPS2021 software (Kingsoft Office Group, China). SPSS 18.0 software was used to analyse the variance in the physiological and biochemical data (The statistical method is *t*-test). Afterwards, the visual imagery was compiled utilizing the Adobe Illustrator CS6 program.

3. Results

3.1. Standard and sample TICs

The experiment analysed 25 amino acids within a 14-min timeframe. The total ion current chromatograms (TICs) for each sample (Fig. S1A) and the extracted ion chromatograms (XICs) (Fig. S1B, C) demonstrated high separation efficiency for individual indices and well-defined peaks. The overlap of TICs acquired from mass spectrometric analysis of various quality control samples revealed significant overlap in the TICs of standard quality control samples (Fig. S1D), and the overlap of TICs (Fig. S1E) with quality control samples data indicated a high degree of concordance in the chromatographic profiles. This finding suggested good signal stability across various time frames for both the mass spectrometry and liquid chromatography systems. A linear R^2 value for each amino acid exceeding 0.99 indicated excellent linearity. Furthermore, a stable relative standard deviation (RSD) below 7% was used for each target compound, indicating the stability and dependability of the method and analytical system. This finding suggested the suitability of the quantitative detection method for sample analysis.

3.2. Comparative analysis of amino acids

The composition of amino acids in the grains of purple rice differed at different stages of grain filling, as shown in Table 1. The 4-Hpro content in the D28 stages was significantly greater than that in the D7, D14, D21, and D35 stages, with increases of 90.5, 85.0, 56.1, and 6.7%, respectively. Additionally, there were significant differences observed between D28 and D7, D14, and D21. The b-Ala content was highest in D35, and compared with that in D7, D14, D21, and D28. GABA reaches its peak content during the D35 stage and is lowest during the D7 stage. The Ala content was highest in D35, and compared with D7, D14, D21, and D28. Arg exhibited its highest content during the D35 stage. The Asn content was highest during the D35 stage and lowest during the D14 stage. Asp and Cys both exhibited their highest levels during the D28 stage and their lowest levels during the D14 stage. The Glu content was highest in D35, and compared with that in D7, D14, D21, and D28. The Gln content was highest in the D35 stage, and compared with the D7, D14, D21, and D28 stages. The Gly content was greatest during the D35 stage and lowest during the D7 stage. The His content was highest in D35. The Ile content was highest in D35, and compared with D7, D14, D21, and D28. The Ser content was greatest in D35. Thr and Try

Table 1
Differences in amino acid content of purple rice grains at five successive stages of grain filling.

Amino acid name	D7	D14	D21	D28	D35
4-Hpro (μg g^{-1})	1.7 ± 0.0c	1.7 ± 0.0c	2.1 ± 0.1b	3.2 ± 0.2a	3.0 ± 0.0a
b-Ala (μg g^{-1})	2.1 ± 0.3e	5.9 ± 0.7d	11.0 ± 1.4c	43.0 ± 1.5b	54.6 ± 1.7a
GABA (μg g^{-1})	49.0 ± 2.4c	69.0 ± 2.1d	54.2 ± 1.6c	69.9 ± 2.7b	75.0 ± 0.7a
Ala (μg g^{-1})	178.3 ± 28.2e	510.5 ± 22.5d	1063.0 ± 39.3c	2849.4 ± 75.3b	3498.3 ± 55.3a
Arg (μg g^{-1})	106.2 ± 20.0d	142.4 ± 13.2 cd	178.4 ± 9.7bc	215.4 ± 13.4ab	220.2 ± 16.3a
Asn (μg g^{-1})	40.8 ± 8.3d	38.7 ± 4.3d	61.2 ± 2.8c	195.6 ± 9.6b	479.6 ± 9.08a
Asp (μg g^{-1})	137.3 ± 23.0c	132.2 ± 4.8c	458.9 ± 3.0b	1075.8 ± 19.7a	962.4 ± 93.0a
Cys (μg g^{-1})	0.9 ± 0.2c	0.8 ± 0.1c	1.0 ± 0.1c	2.8 ± 0.2a	1.9 ± 0.3b
Glu (μg g^{-1})	181.1 ± 26.7d	343.6 ± 41.6d	999.2 ± 37.7c	1984.4 ± 92.0b	2384.9 ± 212.9a
Gln (μg g^{-1})	150.1 ± 25.7d	222.1 ± 20.9d	622.1 ± 30.4c	939.9 ± 166.5b	1923.7 ± 27.7a
Gly (μg g^{-1})	39.2 ± 3.4d	63.5 ± 6.8 cd	93.7 ± 7.0c	366.0 ± 23.8b	503.8 ± 8.5a
His (μg g^{-1})	15.3 ± 3.0d	50.8 ± 5.0c	56.6 ± 4.1c	150.5 ± 9.4b	189.74 ± 4.6a
Ile (μg g^{-1})	104.0 ± 11.7e	241.8 ± 13.4d	382.6 ± 6.7c	924.0 ± 58.0b	1231.0 ± 61.1a
Leu (μg g^{-1})	18.6 ± 1.8e	54.6 ± 2.7d	61.7 ± 2.8c	166.2 ± 6.2b	246.3 ± 8.4a
Lys (μg g^{-1})	65.4 ± 12.2e	195.7 ± 15.0d	299.6 ± 11.9c	695.0 ± 22.1b	824.6 ± 36.3a
Met (μg g^{-1})	3.5 ± 0.1e	5.8 ± 0.4d	9.0 ± 0.5c	22.6 ± 1.2b	34.4 ± 1.3a
Orn (μg g^{-1})	6.2 ± 1.1d	15.2 ± 0.7c	16.5 ± 0.0c	23.3 ± 1.4b	49.3 ± 1.0a
Phe (μg g^{-1})	37.0 ± 4.7e	79.6 ± 5.5d	152.5 ± 3.9c	351.4 ± 7.8b	415.1 ± 5.6a
Pro (μg g^{-1})	15.8 ± 1.7e	29.6 ± 2.0d	68.9 ± 2.1c	178.4 ± 6.0b	220.5 ± 1.6a
Ser (μg g^{-1})	83.4 ± 10.2e	184.7 ± 17.1d	289.2 ± 6.2c	662.1 ± 30.0b	978.1 ± 25.0a
Thr (μg g^{-1})	29.7 ± 5.4d	72.3 ± 6.2c	106.8 ± 2.7c	352.2 ± 12.9b	647.9 ± 27.8a
Try (μg g^{-1})	12.9 ± 1.0d	92.5 ± 3.0c	96.2 ± 5.1c	141.9 ± 5.6b	248.4 ± 6.9a
Tyr (μg g^{-1})	16.1 ± 3.1e	37.0 ± 3.6d	91.4 ± 2.7c	270.0 ± 11.6b	365.1 ± 3.5a
Val (μg g^{-1})	43.3 ± 4.9e	96.5 ± 4.4d	116.6 ± 5.4c	298.0 ± 3.5b	440.9 ± 12.0a
Tau (μg g^{-1})	7.6 ± 1.5d	13.6 ± 0.9 cd	31.9 ± 3.0c	74.3 ± 11.9a	46.3 ± 11.2b

The lowercase letters on the same line represent significant statistical differences at the 0.05 level. 4-Hpro, 4-hydroxyproline; b-Ala, beta-alanine; GABA, gamma-aminobutyric acid; Ala, L-alanine; Arg, L-arginine; Asn, L-asparagine; Asp, L-aspartic acid; Cys, L-cysteine; Glu, L-glutamic acid; Gln, L-glutamine; Gly, L-glycine; His, L-histidine; Ile, L-isoleucine; Leu, L-leucine; Lys, L-lysine; Met, L-methionine; Orn, L-ornithine; Phe, *L*-phenylalanine; Pro, L-proline; Ser, L-serine; Thr, L-threonine; Trp, L-tryptophan; Tyr, L-tyrosine; Val, L-valine; Tau, taurine. After 7 days (D7), 14 days (D14), 21 days (D21), 28 days (D28), and 35 days (D35) following the filling of the rice grains. The data are shown as the mean ± s. d. ($n = 3$).

exhibited the highest levels during the D35 stage and the lowest level during the D7 stage. The content of all amino acids was the highest in the fifth phase of grain filling (D35), except for 4-Hpro, Asp, Cys, Tau.

3.3. Differential analysis of several nutritional components

The nutritional components of purple rice grains vary across different stages of grain filling (Table 2). The CP content in D7 and D14 was significantly greater than that in D7, D14, and D21. The highest content of AsA was found in D35. The TS content was greater in D28 and D35, with the lowest TS content observed in D14. CAROT content D35 increased significantly by 149.8, 175.0, 145.3, and 121.1% compared to D7, D14, D21, and D28, respectively. The VB9 content was highest in D35. The CF content was greater in D7 and D21, with the lowest CF content observed in D35. The crude protein content was highest in D7. Similarly, the C3OG content was greatest in D35. P3G was either undetected or present at relatively low levels during the D7 stage but showed the highest content during D35. ATP exhibited its highest content during the D21 stage and its lowest content during the D28 stage.

3.4. Differential analysis of mineral elements

The mineral element content in purple rice grains varied across different growth stages (Table 3). The Fe, Mn, and Ca contents in D35 and D28 were significantly greater than those in D7, D14, and D21. The contents of Fe, Mn, and Ca in D35 were 46.22, 31.01, and 46.39% greater, respectively, than those in D7, D14, and D21. The Fe, Mn, and Ca contents in D28 were 36.08, 21.93, and 36.24% greater, respectively, than those in D7, D14, and D21. The Zn, Cu, and Mg contents in D35 were significantly greater than those in D7, D14, and D21. Specifically, the Zn content in D35 was 101.57, 94.49, and 67.83% greater than that in D7, D14, and D21, respectively. Similarly, the Cu content in D35 was highest. The Mg content in D35 was 19.78, 26.67, and 19.52% greater than that in D7, D14, and D21, respectively. Additionally, the Se content in D35 was significantly greater than that in D7, D14, D21, and D28. This study revealed that the contents of various mineral elements increase with the continuous filling of purple rice grains.

Table 2
Differences in main nutrient components of purple rice at five successive stages of grain filling.

Indicator name	D7	D14	D21	D28	D35
ASA (μg g^{-1})	53.9 ± 1.0b	76.7 ± 8.2b	324.4 ± 31.0b	332.1 ± 15.2b	1301.9 ± 186.9a
TS (mg g^{-1})	40.5 ± 7.9c	36.7 ± 7.0c	68.2 ± 8.8b	98.8 ± 5.4a	92.4 ± 12.2a
CAROT (μg g^{-1})	77.4 ± 1.6b	70.3 ± 0.6b	78.8 ± 3.1b	87.5 ± 1.2b	193.4 ± 34.3a
VB9 (μg g^{-1})	0.7 ± 0.0c	1.4 ± 0.1b	1.4 ± 0.0b	1.6 ± 0.0b	5.6 ± 0.2a
CF (%)	2.9 ± 0.0a	2.5 ± 0.0ab	3.0 ± 0.0a	2.4 ± 0.0ab	2.0 ± 0.0ab
CP (g kg^{-1})	132.8 ± 21.2a	108.8 ± 3.9ab	88.1 ± 0.7b	83.2 ± 4.1b	81.1 ± 1.0b
C3OG (μg g^{-1})	9.1 ± 3.4c	24.8 ± 0.0c	1141.5 ± 173.1bc	1759.4 ± 570.3b	5398.3 ± 945.5a
P3G (μg g^{-1})	0.00	4.4 ± 1.4c	121.3 ± 30.9bc	198.1 ± 109.5b	489.7 ± 92.5a
ATP (μg g^{-1})	16.7 ± 0.4ab	16.8 ± 0.1a	17.7 ± 0.2a	11.8 ± 0.6c	15.8 ± 0.3b

AsA, ascorbic acid; TS, total sugar; CAROT, carotenoid; VB9, vitamin B9; CF, Crude fat; CP, crude protein; C3OG, cyanidin-3-O-glucoside; P3G, peonidin 3-glucoside; ATP, adenosine triphosphate. After 7 days (D7), 14 days (D14), 21 days (D21), 28 days (D28), and 35 days (D35) following the filling of the rice grains. The data are shown as the mean ± s.d. (n = 3).

Table 3
Differences in seven mineral elements of purple rice at five successive stages of grain filling.

Indicator name	D7	D14	D21	D28	D35
Fe (mg kg^{-1})	68.5 ± 13.9a	76.4 ± 15.3a	68.4 ± 2.5a	93.2 ± 0.4a	100.1 ± 10.7a
Mn (mg kg^{-1})	27.5 ± 1.2b	30.0 ± 1.2b	45.7 ± 4.0b	112.7 ± 20.0a	127.7 ± 10.2a
Zn (mg kg^{-1})	17.9 ± 2.2c	18.5 ± 2.8c	21.5 ± 1.7bc	31.1 ± 6.1ab	36.0 ± 3.9a
Cu (mg kg^{-1})	3.9 ± 0.1a	5.1 ± 1.7a	5.1 ± 1.1a	5.7 ± 0.3a	13.2 ± 2.7a
Ca (mg kg^{-1})	335.4 ± 12.2c	454.5 ± 201.4c	403.6 ± 20.9c	749.2 ± 95.6b	1037.0 ± 43.8a
Mg (mg kg^{-1})	1180.6 ± 48.2ab	1116.4 ± 60.6b	1183.2 ± 51.0ab	1350.9 ± 142.4ab	1414.2 ± 105.4a
Se (mg kg^{-1})	0.83 ± 0.14ab	0.14 ± 0.02b	0.05 ± 0.03ab	3.81 ± 0.61a	1.14 ± 0.05ab

Fe, iron; Mn, manganese; Zn, Zinc; Cu, Copper; Ca, Calcium; Mg, Magnesium; Se, selenium. After 7 days (D7), 14 days (D14), 21 days (D21), 28 days (D28), and 35 days (D35) following the filling of the rice grains. The data are shown as the mean ± s.d. ($n = 3$).

3.5. The relationships between amino acids and metabolites examined through correlation analysis

By referring to our previously published data on metabolites in five stages (Xiong et al., 2023), we performed a correlation analysis to examine the relationship between differentially abundant metabolites and amino acid levels in various stages of purple rice. The analysis explored the relationships between the levels of the identified metabolites citric acid, L-isoleucine, trigonelline, and L-glutamate and amino acid (Fig. 1). Citric acid, L-isoleucine, L-glutamate and trigonelline levels were significantly negatively correlated with most amino acids.

3.6. Correlation analysis of major nutrients and metabolites

We performed correlation analysis between the levels of differentially abundant metabolites and major nutrient components during purple rice grain filling at various stages using our previously published metabolite data from five stages (Xiong, Zhang, et al., 2023). The analysis explored the relationships between the identified metabolites citric acid, L-isoleucine, trigonelline, and L-glutamate and the contents of major nutrient components (Fig. S2). CP and CF levels were significantly positively correlated with citric acid, L-isoleucine, and trigonelline levels. The citric acid content was negatively correlated with P3G, TS, CAROT, VB9, and C3OG levels. P3G, ASA, TS, CAROT, VB9, and C3OG levels exhibited significant negative correlations with L-isoleucine, trigonelline, and L-glutamate levels.

3.7. Correlation analysis of mineral elements and metabolites

In relation to our previously released metabolite information throughout five stages (Xiong, Zhang, et al., 2023), we performed a correlation examination between differentially abundant metabolites and the mineral components Fe, Mn, Zn, Cu, Ca, Mg, and Se at various stages of purple rice grain filling. The correlations between the identified metabolites (citric acid, L-isoleucine, trigonelline, and L-glutamate) and the mineral elements Fe, Mn, Zn, Cu, Ca, Mg, and Se were investigated (Fig. S3). Fe, Mn, Zn, Cu, Ca, Mg, and Se levels exhibited significant negative correlations with trigonelline, L-isoleucine, and L-glutamate levels. Citric acid was significantly negatively correlated with Fe, Zn, Ca, Mg, and Se levels.

Fig. 1. Correlation analysis of amino acid with DMs. The right side shows the names of DMs, and the bottom indicates amino acid. Each grid represents the correlation between the two attributes, and different colours represent the sizes of the correlation coefficients between the attributes. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. The same as below.

3.8. Nutritional component biomarkers at various stages in purple rice

Correlation analysis between the identified metabolites and amino acids revealed significant correlations with citric acid, L-isoleucine, trigonelline, and L-glutamate levels, indicating their potential as promising biomarkers for metabolism. Moreover, correlation analysis of

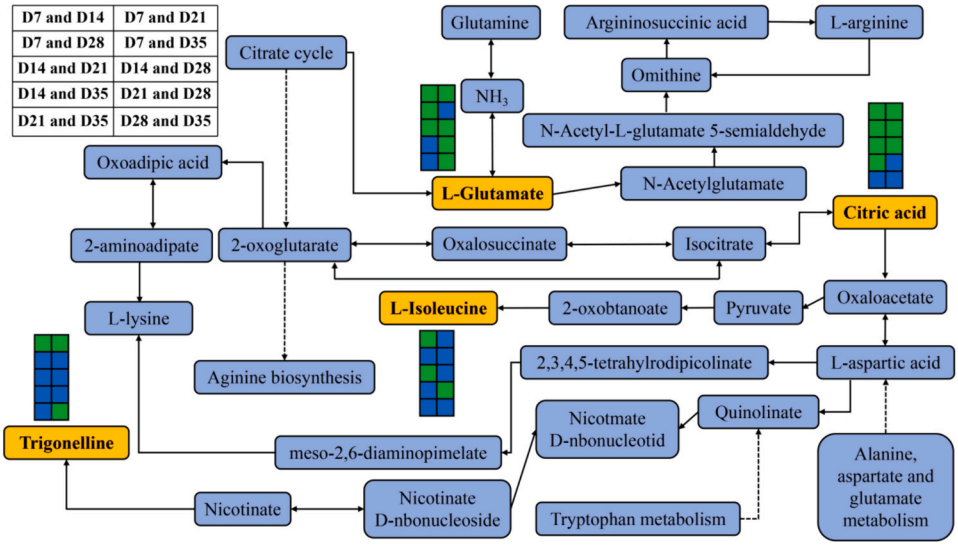


Fig. 2. The key metabolites in metabolic pathways in pairwise comparisons. The key metabolites are shown in the orange rectangle. Small blue rectangles indicate significant downregulation of the metabolite content; and small green rectangles indicate no significant difference in the metabolite content. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the recognized metabolites and key nutrient constituents revealed noteworthy associations with citric acid, L-isoleucine, trigonelline, and L-glutamate levels. Moreover, the correlations among the detected metabolites and Fe, Mn, Zn, Cu, Ca, Mg, and Se levels demonstrated a significantly strong correlation with trigonelline, L-isoleucine, and L-glutamate levels, suggesting their potential as promising metabolic indicators. The primary potential metabolic biomarkers were trigonelline, L-isoleucine, L-glutamate, and citric acid, as indicated by the three sets of correlation analyses, which demonstrated their close association with nutrient components. For detailed information on the metabolite markers, please refer to Table S4. We generated a schematic diagram of the synthesis of the nutritional metabolites citric acid, L-isoleucine, trigonelline, and L-glutamate (Fig. 2).

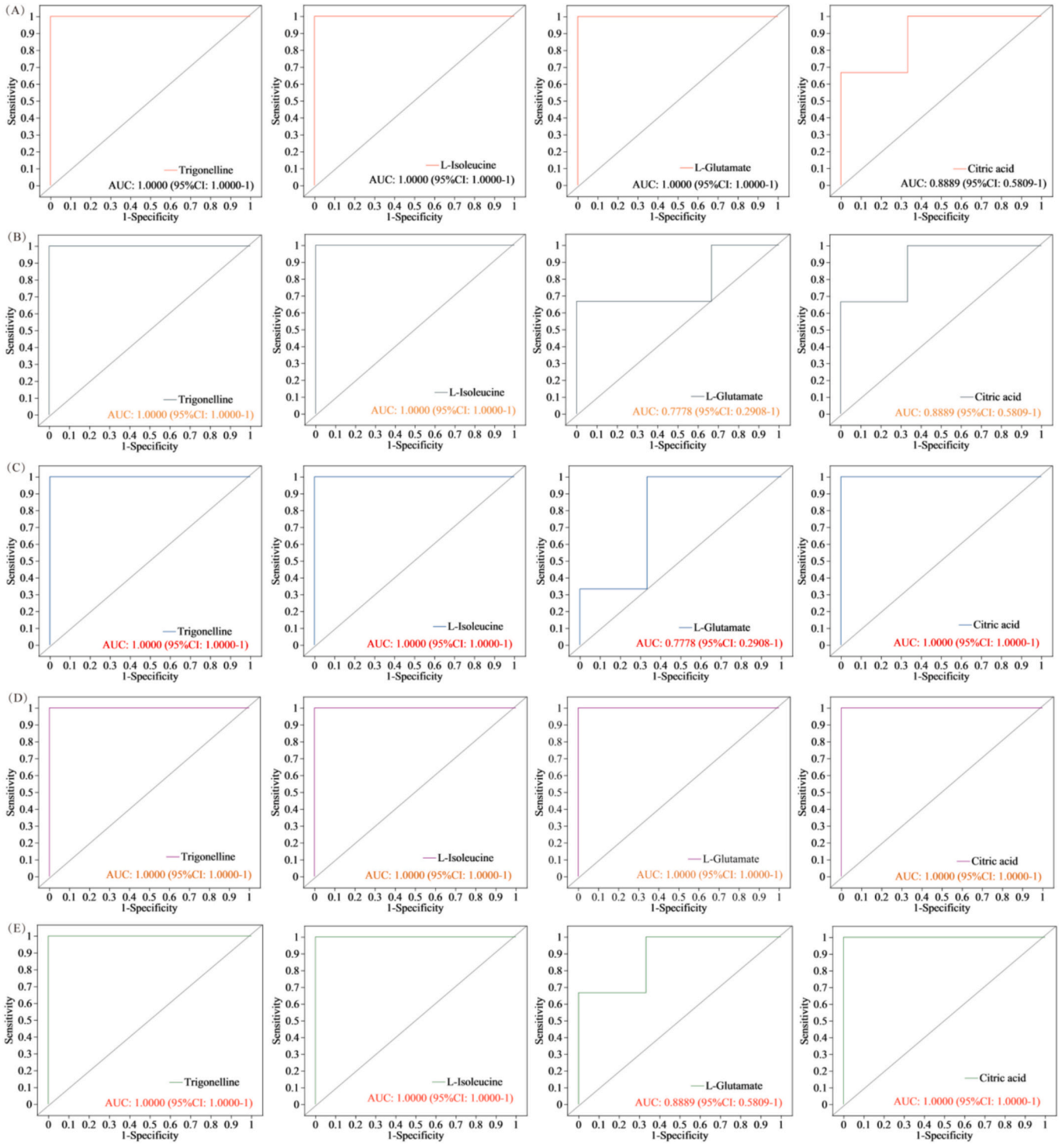


Fig. 3. Receiver operating characteristic (ROC) analysis. (A) ROC analysis of trigonelline, L-isoleucine, L-glutamate, citric acid between D14 and D7; (B) ROC analysis of trigonelline, L-isoleucine, L-glutamate, citric acid between D21 and D7; (C) ROC analysis of trigonelline, L-isoleucine, L-glutamate, citric acid between D28 and D7; (D) ROC analysis of trigonelline, L-isoleucine, L-glutamate, citric acid between D35 and D7; (E) ROC analysis of trigonelline, L-isoleucine, L-glutamate, citric acid between D21 and D14.

3.9. Receiver operating characteristic (ROC) curve analysis

The areas under the curve (AUCs) of trigonelline, L-isoleucine, L-glutamate, and citric acid between D14 and D7 were 1, 1, 1 and 0.8889, respectively (Fig. 3A). The AUCs of trigonelline, L-isoleucine, L-glutamate, and citric acid between D21 and D7 were 1, 1, 0.7778, and 0.8889, respectively (Fig. 3B). The AUCs of trigonelline, L-isoleucine, L-glutamate, and citric acid between D28 and D7 were 1, 1, 0.7778 and 1, respectively (Fig. 3C). The AUCs of trigonelline, L-isoleucine, L-glutamate, and citric acid between D35 and D7 were 1, 1, 1 and 1, respectively (Fig. 3D). The AUCs of trigonelline, L-isoleucine, L-glutamate, and citric acid between D21 and D14 were 1, 1, 0.8889 and 1, respectively (Fig. 3E). The AUCs of trigonelline, L-isoleucine, L-glutamate, and citric acid between D28 and D14 were 1, 1, 1 and 1, respectively (Fig. S4A). The AUCs of trigonelline, L-isoleucine, L-glutamate, citric acid between D35 and D14 were 1, 1, 1 and 1, respectively (Fig. S4B). The AUCs of trigonelline, L-isoleucine, L-glutamate, and citric acid between D28 and D21 were 1, 1, 1 and 1, respectively (Fig. S4C). The AUCs of trigonelline, L-isoleucine, L-glutamate, and citric acid between D35 and D21 were 1, 1, 1 and 1, respectively (Fig. S4D). The AUCs of trigonelline, L-isoleucine, L-glutamate, and citric acid for D35 and D28 were 1, 1, 1 and 1, respectively (Fig. S4E). For AUC values >0.5, a higher AUC value indicates better diagnostic performance. AUC values ranging from 0.5 to 0.7 indicate a lack of precision, whereas values between 0.7 and 0.9 imply a moderate level of accuracy. AUC values exceeding 0.9 indicate high accuracy.

4. Discussion

The nutritional value of rice protein is determined by fluctuations in amino acid composition, particularly the quantities of vital amino acids present during various growth phases following the maturation of purple rice grains. This ratio serves as a significant indicator of the quality of rice (Kawakatsu & Takaiwa, 2019; Xiong, Zhang, et al., 2023). During the initial stage of grain filling, rice primarily produces starch, resulting in a significant decrease in the amino acid content of each grain in the early grain filling stage (D7) compared to the later stage (D35) (Table 1) (Xiong, Zhang, et al., 2023). This suggests that as the grain-filling stage progresses in rice, there is a continuous accumulation of amino acids in the grains. The metabolites trigonelline, L-isoleucine, L-glutamate, and citric acid exhibited significant negative correlations with most amino acids (Fig. 1). In all the comparison groups, trigonelline, L-isoleucine, L-glutamate, and citric acid were present in downregulated metabolic pathways (Fig. 2). Furthermore, these findings confirmed the conclusion that as the duration of the rice grain-filling stage increased, the concentration of amino acids in the grains increased. Additionally, in comparisons across different stages after rice grain filling, trigonelline, L-isoleucine, L-glutamate, and citric acid exhibited favourable ROC diagnostic performance (Fig. 3). Among them, L-isoleucine and L-glutamate are amino acid-related metabolites (Table S4). These four metabolites are crucial compounds in amino acid synthesis pathways (Fig. 2). Therefore, trigonelline, L-isoleucine, L-glutamate, and citric acid can be regarded as potential metabolic biomarkers for the accumulation of amino acids during the grain-filling process.

Anthocyanins such as P3G and C3OG have demonstrated potential for preventing and promoting health in individuals with metabolic disorders such as diabetes, obesity, and hyperlipidaemia (Dwivedi et al., 2022; Santos de Lima et al., 2023). During the later stages of grain filling, the concentrations of P3G and C3OG exhibited a notable increase in comparison to those in the initial stages (Table 2). AsA has diverse biological functions, including the elimination of harmful radicals and toxins, the ability to act as a powerful antioxidant within the human body, and the ability to mediate inflammatory responses through multiple mechanisms (Lamontagne et al., 2022). Current research suggests that vitamin C can be used to treat inflammatory diseases and improve the prognosis of critically ill patients (Colunga Biancatelli, Berrill, & Marik, 2020; Du et al., 2022). During the D35 stage, the concentration of AsA in purple rice was notably greater than that in D7(Table 2). Carotenoids serve as the primary source of vitamin A in the human body but also possess antioxidant, immunoregulatory, and antiaging properties (Beydoun et al., 2020; Rey, Zacarías, & Rodrigo, 2020). VB9, an essential vitamin for cellular growth and reproduction, is highlighted. Its deficiency has direct implications for conditions such as neural tube defects and megaloblastic anaemia (Cabrera et al., 2019). D35 had considerably greater amounts of carotenoids and VB9 than did D7, D14, D21, and D28 (Table 2). Citric acid was negatively correlated with P3G, TS, CAROT, VB9, and C3OG (Fig. S2). Critic acid, known for its antioxidative properties, also plays a role in various biological processes, such as energy metabolism (Liu, Du, & Chen, 2020; Tahjib-Ul-Arif et al., 2021). Citric acid plays a crucial role in amino acid synthesis (Fig. 2). The downregulation of citric acid metabolites indirectly validated the increases in P3G, TS, CAROT, VB9, and C3OG levels. Further ROC curve analysis revealed that the AUC of citric acid in all stages was >0.8889 (Fig. 3), indicating good diagnostic performance. As the duration of purple rice grain filling progresses, AsA, CAROT, VB9, P3G, and C3OG accumulate rapidly. These findings mutually corroborate our previously published conclusion regarding the high total antioxidant capacity of late-stage filled purple glutinous rice (Xiong, Zhang, et al., 2023).

Minerals are essential elements for the human body that cannot be produced or synthesized internally. To address the problems of micronutrient deficiencies and nutritional imbalances in Asian populations, the optimal strategy is to grow mineral nutrient-enriched rice (Singh, & Vanlalsanga., Mehta, S. K., Singh, Y. T., 2022). The levels of Fe, Mn, Zn, Cu, Ca, Mg, and Se in the purple rice plants exhibited variations throughout the different postfilling stages, suggesting diverse abilities to accumulate mineral components. The Fe, Mn, and Ca contents of D35 and D28 were significantly greater than those of D7, D14, and D21 (Table 3). This indicates a continuous increase in the content of various mineral elements as the purple rice grains mature. Trigonelline, L-isoleucine, and L-glutamate were significantly negatively correlated with Fe, Mn, Zn, Cu, Ca, Mg, and Se levels (Fig. S3). Citric acid was significantly negatively correlated with Fe, Zn, Ca, Mg, and Se (Fig. S3). Therefore, trigonelline, L-isoleucine, L-glutamate, and citric acid can be considered potential metabolic biomarkers for the accumulation of mineral elements during grain filling. Regulating these four potential biomarkers could offer a new approach for cultivating high-nutrient purple rice enriched in mineral elements.

5. Conclusion

Comprehensive analysis suggested that L-isoleucine, L-glutamate, citric acid, and trigonelline could serve as potential biomarkers for nutritional metabolism. This research provides a theoretical basis for the use of metabolomics in breeding and provides fresh perspectives on creating purple rice grains with high levels of amino acids, antioxidants, and minerals for enhanced nutritional value. In the future, a comprehensive approach involving modern molecular biology, metabolomics, molecular marker-assisted selection, and other scientific methods should be utilized to analyse and breed new rice with high nutritional content. This approach aims to meet the demand for healthier lifestyles among people.

CRediT authorship contribution statement

Qiangqiang Xiong: Writing – original draft, Investigation. **Yanyao Lu:** Data curation. **Wenfei Gu:** Investigation. **Yu Zhang:** Investigation, Formal analysis. **Ao Li:** Formal analysis. **Shuo Cai:** Writing – review & editing. **Nianbing Zhou:** 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.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101528.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by Zhenjiang Jinshan Talents Industry Strong City Leading Talent Introduction Plan (Zhenjiang Talent Office [2021] No. 1), Jiangsu Province Seed Industry Revitalization Project [JBGS(2021)036], the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

References

Bedhiafi, T., Inchakalody, V. P., Fernandes, Q., Mestiri, S., Billa, N., Uddin, S., … Dermime, S. (2022). The potential role of vitamin C in empowering cancer immunotherapy. *Biomedicine and Pharmacother, 146*, Article 112553. https://doi.org/10.1016/j.biopha.2021.112553

Beydoun, M. A., Canas, J. A., Fanelli-Kuczmarski, M. T., Maldonado, A. I., Shaked, D., Kivimaki, M., Evans, M. K., & Zonderman, A. B. (2020). Association of antioxidant vitamins a, C, E and carotenoids with cognitive performance over time: A cohort study of middle-aged adults. *Nutrients, 12*, 3558. https://doi.org/10.3390/nu12113558

Cabrera, R. M., Souder, J. P., Steele, J. W., Yeo, L., Tukeman, G., Gorelick, D. A., & Finnell, R. H. (2019). The antagonism of folate receptor by dolutegravir: Developmental toxicity reduction by supplemental folic acid. *Aids, 33*(13), 1967–1976. https://doi.org/10.1097/QAD.0000000000002289

Colunga Biancatelli, R. M., Berrill, M., & Marik, P. E. (2020). The antiviral properties of vitamin C. *Expert Review of Anti- infective Therapy, 18*(2), 99–101. https://doi.org/10.1080/14787210.2020.1706483

Determination of fat in food products of the national standard for food safety. (2017). National Standard of the People's Republic of China. *GB*, 5009.6–2016. https://www.chinesestandard.net/PDF/English.aspx/GB5009.6-2016.

Determination of multiple elements in food of the national standard of food safety system. (2017). National Standard of the People's Republic of China GB 5009.268–2016. https://www.chinesestandard.net/PDF/English.aspx/GB5009.268-2016.

Determination of protein in food of the national standard for food safety. (2017). National Standard of the People's Republic of China, GB 5009.5–2016. https://www.chinesestandard.net/PDF/English.aspx/GB5009.5-2016.

Determination of total saccharide in edible mushroom. (2009). National Standard of the People's Republic of China, GB/T 15672-2009. https://www.chinesestandard.net/PDF/English.aspx/GBT15672-2009.

Du, Y. T., Long, Y., Tang, W., Liu, X. F., Dai, F., & Zhou, B. (2022). Prooxidative inhibition against NF-κB-mediated inflammation by pharmacological vitamin C. *Free Radicial Biology and Medicine, 180*, 85–94. https://doi.org/10.1016/j.freeradbiomed.2022.01.007

Dwivedi, S. L., Mattoo, A. K., Garg, M., Dutt, S., Singh, B., & Ortiz, R. (2022). Developing germplasm and promoting consumption of anthocyanin-rich grains for health benefits. *Frontiers in Sustainable Food Systems, 6*, Article 867897. https://doi.org/10.3389/fsufs.2022.867897

Gu, W. F., Peng, Y. H., Wang, R. Z., Wang, R. N., Wu, H., Zhu, J. Y., … Xiong, Q. Q. (2023). Comparison of metabolites and main nutritional components between uncooked and cooked purple rice. *Metabolites, 13*(9), 1018. https://doi.org/10.3390/metabo13091018

Huang, M., Liao, C., Xie, J., Chen, J., & Cao, F. (2023). Lysine content and its relationship with protein content in indica rice landraces of China. *Food Chemistry: X, 17*, Article 100549. https://doi.org/10.1016/j.fochx.2022.100549

Kawakatsu, T., & Takaiwa, F. (2019). 4-Rice proteins and essential amino acids. *AACC International Press*, 109–130.

Kowsalya, P., Sharanyakanth, P. S., & Mahendran, R. (2022). Traditional rice varieties: A comprehensive review on its nutritional, medicinal, therapeutic and health benefit potential. *Journal of Food Composition and Analysis, 114*, Article 104742. https://doi.org/10.1016/j.jfca.2022.104742

Lamontagne, F., Masse, M. H., Menard, J., Sprague, S., Pinto, R., Heyland, D. K., & Adhikari, N. (2022). Intravenous vitamin C in adults with sepsis in the intensive care unit. *New England Journal of Medicine, 386*(25), 2387–2398. https://doi.org/10.1056/NEJMoa2200644

Liu, K. L., Du, R. F., & Chen, F. S. (2020). Stability of the antioxidant peptide SeMet-pro-Ser identified from selenized brown rice protein hydrolysates. *Food Chemistry, 319*, Article 126540. https://doi.org/10.1016/j.foodchem.2020.126540

Mohidem, N. A., Hashim, N., Shamsudin, R., & Che Man, H. (2022). Rice for food security: Revisiting its production, diversity, Rice milling process and nutrient content. *Agriculture, 12*(6), 741. https://doi.org/10.3390/agriculture12060741

Panda, D., Rani, K., Behera, P. K., Pradhan, B., & Lenka, S. K. (2023). Nutritional diversity and food potential of indigenous pigmented rice landraces from Koraput regions of eastern Ghats. *Discover Food, 3*(1), 16. https://doi.org/10.1007/s44187-023-00057-5

Pushpa, R., Sassikumar, D., Suresh, R., & Iyanar, K. (2022). Evaluation of nutritional and grain quality diversity in rice (*Oryza sativa* L.) germplasm based on multivariate analysis. *Electronic journal of. Plant Breeding, 13*(4), 1187–1197. https://doi.org/10.37992/2022.1304.171

Rey, F., Zacarías, L., & Rodrigo, M. J. (2020). Carotenoids, vitamin c, and antioxidant capacity in the peel of mandarin fruit in relation to the susceptibility to chilling injury during postharvest cold storage. *Antioxidants, 9*(12), 1296. https://doi.org/10.3390/antiox9121296

Sangma, H. C., & Parameshwari, S. (2023). Health benefits of black rice (*Zizania aqatica*)-a review. *Materials Today: Proceedings, 80*, 3380–3384. https://doi.org/10.1016/j.matpr.2021.07.257

Santos de Lima, K., Schuch, F. B., Camponogara Righi, N., Chagas, P., Hemann Lamberti, M., Puntel, G. O., … Ulisses Signori, L. (2023). Effects of the combination of vitamins C and E supplementation on oxidative stress, inflammation, muscle soreness, and muscle strength following acute physical exercise: meta-analyses of randomized controlled trials. *Critical Reviews in Food Science and Nutrition, 63*(25), 7584–7597. https://doi.org/10.1080/10408398.2022.2048290

Sarker, U., & Oba, S. (2021). Color attributes, betacyanin, and carotenoid profiles, bioactive components, and radical quenching capacity in selected Amaranthus gangeticus leafy vegetables. *Scientific Reports, 11*(1), 11559. https://doi.org/10.1038/s41598-021-91157-8

Sedeek, K., Zuccolo, A., Fornasiero, A., Weber, A. M., Sanikommu, K., Sampathkumar, S., … Mahfouz, M. M. (2023). Multi-omics resources for targeted agronomic improvement of pigmented rice. *Nature Food, 4*(5), 366–371. https://doi.org/10.1038/s43016-023-00742-9

Shi, Y. H., Zhang, Y. Y., Sun, Y. Y., Xie, Z. Y., Luo, Y., Long, Q. Y., … Luo, J. (2023). Natural variations of OsAUX5, a target gene of *OsWRKY78*, control the neutral essential amino acid content in rice grains. *Molecular Plant, 16*(2), 322–336. https://doi.org/10.1016/j.molp.2022.12.013

Singh, S. P., & Vanlalsanga., Mehta, S. K., Singh, Y. T.. (2022). New insight into the pigmented rice of Northeast India revealed high antioxidant and mineral compositions for better human health. *Heliyon, 8*(8), Article e10464. https://doi.org/10.1016/j.heliyon.2022.e10464

Tahjib-Ul-Arif, M., Zahan, M. I., Karim, M. M., Imran, S., Hunter, C. T., Islam, M. S., … Murata, Y. (2021). Citric acid-mediated abiotic stress tolerance in plants. *International Journal of Molecular Sciences, 22*(13), 7235. https://doi.org/10.3390/ijms22137235

Tai, L., Huang, S., Zhao, Z., & Huang, G. (2021). Chemical composition analysis and antioxidant activity of black rice pigment. *Chemical Biology & Drug Design, 97*(3), 711–720. https://doi.org/10.1111/cbdd.13806

Wisetkomolmat, J., Arjin, C., Satsook, A., Seel-audom, M., Ruksiriwanich, W., Prom-u-Thai, C., & Sringarm, K. (2022). Comparative analysis of nutritional components and phytochemical attributes of selected Thai Rice bran. *Frontiers in Nutrition, 9*, Article 833730. https://doi.org/10.3389/fnut.2022.833730

Xiong, Q. Q., Sun, C. H., Li, A., Zhang, J., Shi, Q., Zhang, Y. H., … Zhu, J. Y. (2022). Metabolomics and biochemical analyses revealed metabolites important for the antioxidant properties of purple glutinous rice. *Food Chemistry, 389*, Article 133080. https://doi.org/10.1016/j.foodchem.2022.133080

Xiong, Q. Q., Wang, R. N., Sun, C. H., Wang, R. Z., Wang, X. Y., Zhang, Y., … Zhu, J. Y. (2023). Metabolites associated with the main nutrients in two varieties of purple rice processed to polished rice. *Metabolites, 13*(1), 7. https://doi.org/10.3390/metabo13010007

Xiong, Q. Q., Zhang, J., Shi, Q., Zhang, Y. H., Sun, C. H., Li, A., … Zhu, J. Y. (2022). The key metabolites associated with nutritional components in purple glutinous rice. *Food Research International, 160*, Article 111686. https://doi.org/10.1016/j.foodres.2022.111686

Xiong, Q. Q., Zhang, J., Sun, C. H., Wang, R. N., Wei, H. Y., He, H. H., … Zhu, J. Y. (2023). Metabolomics revealed metabolite biomarkers of antioxidant properties and flavonoid metabolite accumulation in purple rice after grain filling. *Food Chemistry: X, 18*, Article 100720. https://doi.org/10.1016/j.fochx.2023.100720

Zhang, S., Ma, Q., Dong, L. H., Jia, X. C., Liu, L., Huang, F., … Zhang, R. F. (2022). Phenolic profiles and bioactivities of different milling fractions of rice bran from black rice. *Food Chemistry, 378*, Article 132035. https://doi.org/10.1016/j.foodchem.2021.132035

Zhou, H., Xia, D., Li, P. B., Ao, Y. T., Xu, X. D., Wan, S. S., … He, Y. Q. (2021). Genetic architecture and key genes controlling the diversity of oil composition in rice grains. *Molecular Plant, 14*(3), 456–469. https://doi.org/10.1016/j.molp.2020.12.001

Zhou, X. Q., Chen, D. G., Guo, J., Chen, P. L., Li, L. J., Chen, K., … Zhang, Z. M. (2022). Genetic improvement of grain quality traits in indica inbred rice cultivars developed in South China during 1956–2020. *Euphytica, 218*, 1–17. https://doi.org/10.1007/s10681-021-02953-2

Zhu, J. Y., Shi, Q., Sun, C. H., Hu, J. L., Zhou, N. B., Wei, H. Y., … Xiong, Q. Q. (2022). Processing affects (decreases or increases) metabolites, flavonoids, black rice pigment, and total antioxidant capacity of purple glutinous rice grains. *Food Chemistry: X, 16*, Article 100492. https://doi.org/10.1016/j.fochx.2022.100492