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Original Research

Metabolite Profiling and Comparative Metabolomics Analysis of Jiaozhou Chinese Cabbage (*Brassica rapa* L. *ssp. pekinensis*) Planted in Different Areas

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Abstract

Background: Chinese cabbage (*Brassica rapa* L. *ssp. pekinensis*) is one of the most popular vegetables in China because of its taste and health benefits. The area of production has obvious effects on the quality of Chinese cabbage. However, metabolite profiling and variations in different production areas are still unclear. **Methods**: Here, widely targeted metabolite analyses based on the ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) approach were performed to study the metabolite profiling of Chinese cabbage planted in the Jiaozhou and Jinan areas. **Results**: A total of 531 metabolites were detected, of which 529 were present in the Chinese cabbage from both areas, 108 were found to be chemicals related to Chinese traditional medicine, and 79 were found to correspond to at least one disease. Chinese cabbage is rich in nutritious substances such as lipids, phenolic acids, amino acids and derivatives, nucleotides and derivatives, organic acids, flavonoids, glucosinolates, saccharides, alcohols, and vitamins. Comparative analysis showed that the metabolic profiles differed between areas, and 89 differentially altered metabolites (DAMs) were characterized. Of these, 78 DAMs showed higher levels in Jinan Chinese cabbage, whereas 11 had higher levels in Jiaozhou Chinese cabbage. Two metabolites, S-(Methyl)glutathione and nicotinic acid adenine dinucleotide, were unique in Jiaozhou Chinese cabbage. Based on Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, the DAMs were enriched into 23 pathways, of which tryptophan metabolism and thiamine metabolism were the significant enrichment pathways. **Conclusions**: This study provides new insights into the metabolite profiles and production areas affecting the metabolite variations of Chinese cabbage, which will be useful for functional Chinese cabbage cultivation.

Keywords: Chinese cabbage; Chinese traditional medicine; KEGG; metabolic

1. Introduction

Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) is native to China and one of Asia's most important vegetables. As a brassica vegetable, Chinese cabbage has many health benefits arising from its nutritional, antioxidant, and anti-cancer properties, which are closely related to the metabolite composition of the vegetable [1]. Therefore, studying the plant's metabolic profile and associated activities is very important.

Chinese cabbage is rich in various nutrients, such as protein, carbohydrates, and cellulose. It was reported that Chinese cabbage contains 0.8–1.7 g of protein and 1.5–3.2 g of carbohydrate per 100 g of food [2]. It is also a good source of minerals and vitamins for humans [3]. The contents of soluble protein, vitamin C, and total soluble sugar are usually detected to evaluate the quality of Chinese cabbage in many studies [4]. However, the metabolite compositions of these nutrients in Chinese cabbage are still unclear. Although the nutritional composition of the different types remains consistent, there is significant variation in their specific content. Furthermore, the nutritional value of Chinese cabbage can be significantly influenced by the climatic and soil conditions present in various planting areas.

Besides nutrient activity, Chinese cabbage also has antioxidant, anti-cancer, and anti-inflammatory biological activities proven by modern pharmacological studies [5]. Chinese cabbage contains many isothiocyanate metabolites, such as sulforaphane and indole-3-carbinol, which function as anti-cancer compounds [6,7]. It was also reported that Chinese cabbage has hypolipidemic activity, and many of its effective components have been detected using the metabolomics method [5]. Flavonoids, widely used as natural commercial foods and colorants due to their potential health benefits, have also been extracted from Chinese cabbage [8]. Although some human disease-related substances

have been identified in Chinese cabbage, comprehensive identification of these metabolites are lacking. Many other health-promoting metabolites are yet to be detected in Chinese cabbage.

Differences in their cultivation areas have affected metabolites in cabbage plants [9]. Jiaozhou cabbage, well-known in China, is called "Jiaobai" and is popular at home and abroad. It has a planting history of more than 1000 years. Jiaozhou Chinese cabbage has a tender quality, white juice, delicious taste, and soft fiber and leaves. The unique microclimate and superior geographical conditions in the Jiaozhou Chinese cabbage production area make the quality of Jiaozhou Chinese cabbage unique [9]. Although the main compounds attributed to the flavor of Jiaozhou Chinese cabbage have been identified using an automatic static headspace GC-MS method [9], there is still no comparative metabolomics analysis for the specific profiling of nutrition and human disease-related metabolites in Jiaozhou Chinese cabbage.

In this study, widely targeted metabolite analyses based on ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) were performed using one of the most popular Chinese cabbage varieties, Gailiangqingza No. 3, to comprehensively study the metabolite profiling of the leafy head of Chinese cabbage. Comparative metabolomics analyses were subsequently conducted between the two different plant areas, Jiaozhou and Jinan. Our work provides valuable information for assessing Chinese cabbage's nutritional and medical value.

2. Materials and Methods

2.1 Plant Materials

Gailiangqingza No. 3 is a variety of Jiazhou cabbage, the most popular Chinese cabbage variety and is of a high quality, disease resistance, with a high yield, and outstanding storage and transportation resistance in the Jiaozhou area, which was used in the study. We planted Gailiangqingza No.3 on August 15th, 2020, in the field of Qingdao Jiaozhou Chinese Cabbage Research Institute (Jiaozhou, Shandong, China) and the field of the Vegetable Research Institute, Shandong Academy of Agricultural Sciences (Jinan, Shandong, China) in terms of climatology and soil composition. Jiaozhou Chinese cabbage (JA) area was located in Anjiagou Village, Jiaozhou City, Qingdao (119°83′97.55″ N, 36°13′92.41″ E). It belongs to the continental climate in the warm temperate semi-humid monsoon region. Its characteristics include sufficient light, rich heat, rain and heat in the same season, four distinct seasons, and a long frost-free period. The daytime temperature in autumn is between 15–24 °C, and the temperature difference between day and night is about 10 °C. The soil is divided into brown, fluvo-aquic, Shajiang black, saline, and paddy soil [10].

Jinan Chinese cabbage (CQ) was located in Pangzhuang Village, Changqing District, Jinan City, Shandong Province (116°73′38.89″N, 36°38′92.29″E). Warm temperate semi-humid continental monsoon climate with four distinct seasons. In autumn, the daytime temperature is 18–28 °C, and the temperature difference between day and night is about 10 °C. The soil is mainly brown loam, brown soil, and sandy terroir. Standard field management was carried out during the plant growth using conventional management in open fields [11], and the tight leafy heads were harvested in November 2020.

Fresh Chinese cabbages were randomly collected from Jiaozhou and Jinan, respectively. After removing the roots and outer leaves, the leafy heads were cut up and mixed evenly. The mixed samples were collected using the quartering method to detect quality-related substances and metabolites. Each sample had three biological replicates, and each repeat included three individual plants. The physical and chemical characteristics of the tested soil are shown in Table 1.

2.2 Detection of Quality-Related Substances in Chinese Cabbage

The contents of soluble protein, crude fiber, dry matter, vitamin C, and total soluble sugar are usually used to evaluate the nutritional quality of Chinese cabbage. The study determined soluble protein content using the Kjeldahl nitrogen method (GB/T 5009. 5. 2016) [12]. Protein was decomposed to produce ammonia reacted with sulfuric acid, producing ammonium sulfate under catalytic and heating conditions. Boric acid absorbs ammonia during alkaline distillation, titrating with sulfuric acid or hydrochloric acid in a typical volumetric solution. The nitrogen content was calculated based on the acid consumption and then multiplied by the conversion coefficient to get the protein content. The crude fiber was determined according to GB/T 5009.10-2003 [13]. The sample's sugar, starch, fructose, and hemicellulose contents were hydrolyzed and eliminated by sulfuric acid. Crude fiber, as the remaining substance, was subjected to an alkali treatment to remove the protein and fatty acids. The contaminants were removed after ashing if they were insoluble in acid and base. The dry matter was determined using direct drying experiments according to GB 5009.3-2016 [14]. The dry weight loss in the sample, including absorbent water, partially crystallized water, and substances that are volatile under these conditions, was calculated using the volatile method using the physical properties of water in food at 101.3 kPa (one atmospheric pressure) and the temperature of 101–105 °C. Then the moisture content was calculated using the weight values before and after. Vitamin C content was determined according to GB/T 5009. 86. 2016 [15]. After dissolving ascorbic acid in the sample with metaphosphoric acid and sonicating it, an ion pair reagent was used as the mobile phase. It was separated using a reverse phase column. L(+)-ascorbic

Table 1. Physical and chemical characteristics of the JA and CQ soil.

Sample name	pH	OM (g/kg)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mn (mg/kg)
CQ-1	7.90	18.20	31.10	66.20	275.00	3.10×10^4	541.00
CQ-2	7.70	17.80	52.20	65.20	319.00	3.32×10^4	558.00
CQ-3	8.10	19.30	60.10	68.20	382.00	2.95×10^4	552.00
Average	7.90	18.43	47.80	66.53	325.33	3.12×10^4	550.33
JA-1	5.10	10.80	54.20	166.00	128.00	6.57×10^3	448.00
JA-2	5.00	13.70	35.00	143.00	150.00	6.99×10^3	618.00
JA-3	5.10	14.90	61.20	182.00	199.00	3.99×10^3	569.00
Average	5.07	13.13	50.13	163.67	159.00	5.85×10^3	545.00

Note: OM, Organic matter; N, Nitrogen; P, Phosphorus; K, Potassium; Ca, Calcium; Mn, Manganese; JA, Jiaozhou Chinese cabbage; CQ, Jinan Chinese cabbage.

acid and D(+)-ascorbic acid were measured directly using a liquid chromatograph with a UV detector (wavelength 245 nm). Following the reduction in L(+)-dehydroascorbate in the samples by L-cysteine solution, the content of L(+)-dehydroascorbic acid was determined by measuring the total amount of L(+)-ascorbic acid with a UV detector (wavelength of 245 nm) or subtracting the L(+) ascorbic acid content measured in the original sample. Total soluble sugar was determined using the Shaffer-Somogyi method [16].

2.3 Qualitative and Quantitative Determination of Metabolites

2.3.1 Sample Preparation and Extraction

The samples were vacuum freeze-dried and ground using a mixer mill (MM 400, Retsch, Haan, Germany) for 1.5 min at 30 Hz. Then, 100 mg of powder was weighed and dissolved in 1.2 mL of 70% methanol. The mixture was swirled once every 30 minutes for 30 seconds, six times in total, and the sample was placed in a 4 °C refrigerator overnight. After centrifugation (12,000 rpm, 10 minutes), undissolved residues were removed, and the retained supernatants were filtered through a microporous membrane (pore size of 0.22 µm) and stored in a sample bottle for UPLC-MS/MS analysis.

2.3.2 UPLC and ESI-Q TRAP-MS/MS Conditions

UPLC-MS/MS analysis was conducted at MetWare Biological Science and Technology Co., Ltd. (Wuhan, China) using a UPLC (SHIMADZU Nexera X2, https://www.shimadzu.com.cn/) and tandem mass spectrometry system (MS/MS) (Applied Biosystems 4500 QTRAP, http://www.appliedbiosystems.com.cn/). The analytical conditions of UPLC and ESI-Q TRAP-MS/MS were set according to Li *et al*. (2019) [17].

2.3.3 Qualitative and Quantitative Determination of Metabolites

The primary and secondary MS data were used for the qualitative analysis of metabolites based on the publicly available metabolite databases and the self-built MetWare database (MWDB) (Wuhan MetWare Biotechnology Co., Ltd., Wuhan, China) [18]. Regarding the qualitative nature of the substance, MWDB and the qualitative substance were carried out according to the secondary spectrum information. During the analysis, the isotope signal, including the repeated signal of K^+ ion, Na^+ ion, NH_4^+ ion, and the compound itself duplicated signals of fragment ions of other larger molecular weight species [18–20]. Information about the obtained data, namely Level: substance identification level; A: the secondary mass spectrometry and RT of the sample substance are consistent with the database substance verification; B: the sample substance Q1, Q3, RT, DP, CE is consistent with the database substance verification; Q1: the parent ion molecular weight of the substance after the electrospray ion source is added to the ion; Q3 (Da): the characteristic fragment ion, Q1, and Q3 are the m/z mass-to-charge ratio data. Furthermore, during the analysis process, the isotopic signals, the repeated signals of K^+, Na^+, and NH_4^+, and the repeated signals of fragment ions that are other larger molecular weight substances were eliminated.

The quantitative analysis of metabolites was carried out using triple quadrupole mass spectrometry in multiple reaction monitoring (MRM) mode. In MRM mode, the target substance's precursor ions (parent ions) were screened first, and then the ions of other molecular weight substances were removed to eliminate interference. Precursor ions were broken into many fragment ions after ionization in the collision chamber. The fragment ions were then filtered using the triple quadrupole filter to select the target fragment ions. At the same time, the non-target ions were eliminated to avoid interference and enhance the accuracy and reproducibility of the quantitative analysis of metabolites. After obtaining the analysis data of metabolites in different samples, the peak areas of all substances were integrated, and the peak areas of the same metabolites in different samples were corrected. The corresponding relative metabolite contents were expressed as chromatographic peak area integrals [21].

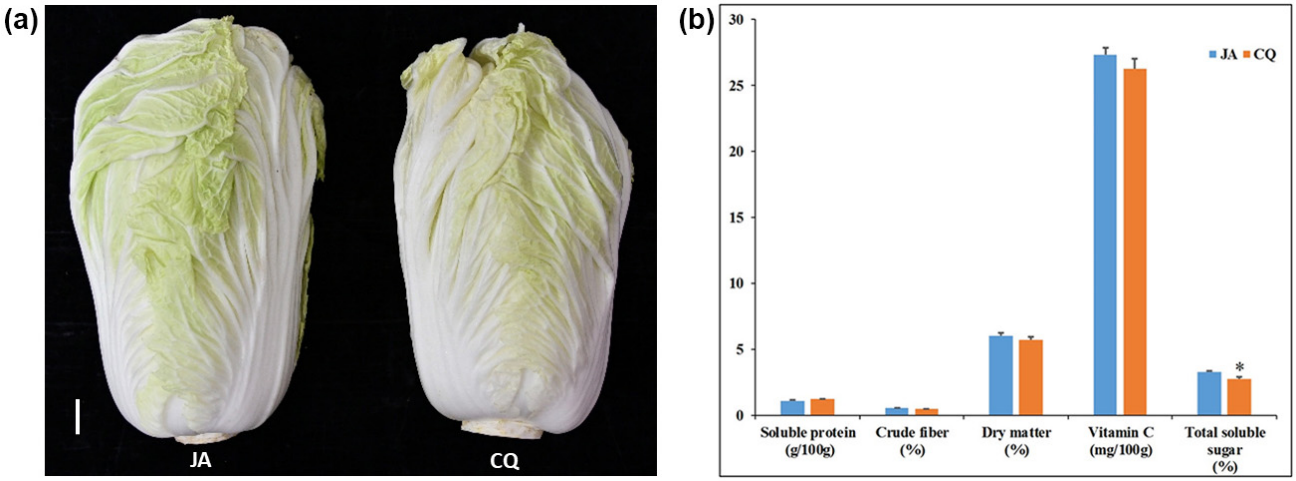

Fig. 1. Quality detection of Chinese cabbage in Jiaozhou (JA) and Jinan (CQ). (a) Leafy head of Chinese cabbage Gailiangqingza No.3 planted in JA and CQ, bar = 5 cm. (b) The contents of soluble protein, crude fiber, dry matter, vitamin C, and total soluble sugars of JA and CQ. *: Difference significance at $p < 0.05$ level according to independent T-test between JA and CQ.

2.4 Statistical Analysis

Hierarchical clustering analysis (HCA), principal component analysis (PCA), and orthogonal partial least squares discriminant analysis (OPLS-DA) of the identified metabolites were performed using the statistics function within R (https://www.r-project.org/), and an independent T-test was conducted using SPSS (Version 13.0., SPSS Inc., Chicago, IL, USA).

Differential Metabolites Analysis

Differential metabolite screening was performed based on the variable importance in projection (VIP) value ≥ 1 of the OPLS-DA model and the $|\log2(\text{fold change})| \geq 1$. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to annotate the identified metabolites and to conduct pathway enrichment analysis. The significantly enriched pathways were considered at a threshold of $p \leq 0.05$.

3. Results and Discussions

3.1 Quality Detection in the Leafy Heads of Chinese Cabbage

The leafy head appearance of Gailiangqingza No.3 had no significant differences between the Chinese cabbages planted in Jinan and Jiaozhou (Fig. 1a). However, the flavor was significantly different between JA and CQ according to the taste assessments of 10 people following previous research [22]. Jiaozhou Chinese cabbage was much crunchier and sweeter based on the testing taste of 10 respondents (**Supplementary Table 1**). We chose 10 people as a representative from 24–56 years old males and females, of which 4 of 10 respondents were male living in Jinan and Qingdao. The study determined the contents of soluble protein, crude fiber, dry matter, vitamin C, and total soluble sugar to assess the quality differences between Chinese JA and CQ. The results showed no significant differences in soluble protein, crude fiber, dry matter, or vitamin C. In contrast, the total soluble sugar content of JA was higher than that of CQ (Fig. 1b). The N and P supply in JA was higher than CQ, illustrating that N and P affected soluble sugar content (Table 1). Our result is in accordance with a previous study which found that the enhancement of total soluble sugar content has a positive correlation to the N and P supply [23]. Similarly, another previous study stated that higher K content in soil significantly reduces the total soluble sugar content [24]. Moreover, the organic matter positively correlated with K absorption, where organic matter considerably promotes the initial fast rate of K adsorption and has more easily accessible adsorption sites for K [25]. We assumed that the lower total soluble sugar content in CQ was related to the soil's N, P, and K content. Furthermore, this may be the reason why JA tasted sweeter than CQ [26].

3.2 Widely Targeted Metabolites Analysis in the Leafy Heads of Chinese Cabbage

3.2.1 Classification of Metabolites Detected in Chinese Cabbage

We further performed a widely targeted metabolite analysis based on UPLC-MS/MS analysis to study the metabolites of Chinese cabbages. There were three biological repeats for each area. Hierarchical clustering analysis (HCA) was used to visualize global metabolite alterations in the Chinese cabbages grown in different areas (Fig. 2). The results showed that the samples were divided into two groups according to the planting area.

A total of 531 and 529 metabolites were detected in JA and CQ, respectively. All the detected metabolites belonged to 11 class I classes and 28 class II classes. In total, 529 metabolites, including 96 lipids, 82 phenolic acids, 80 amino acids and derivatives, 45 alkaloids, 40 nucleotides

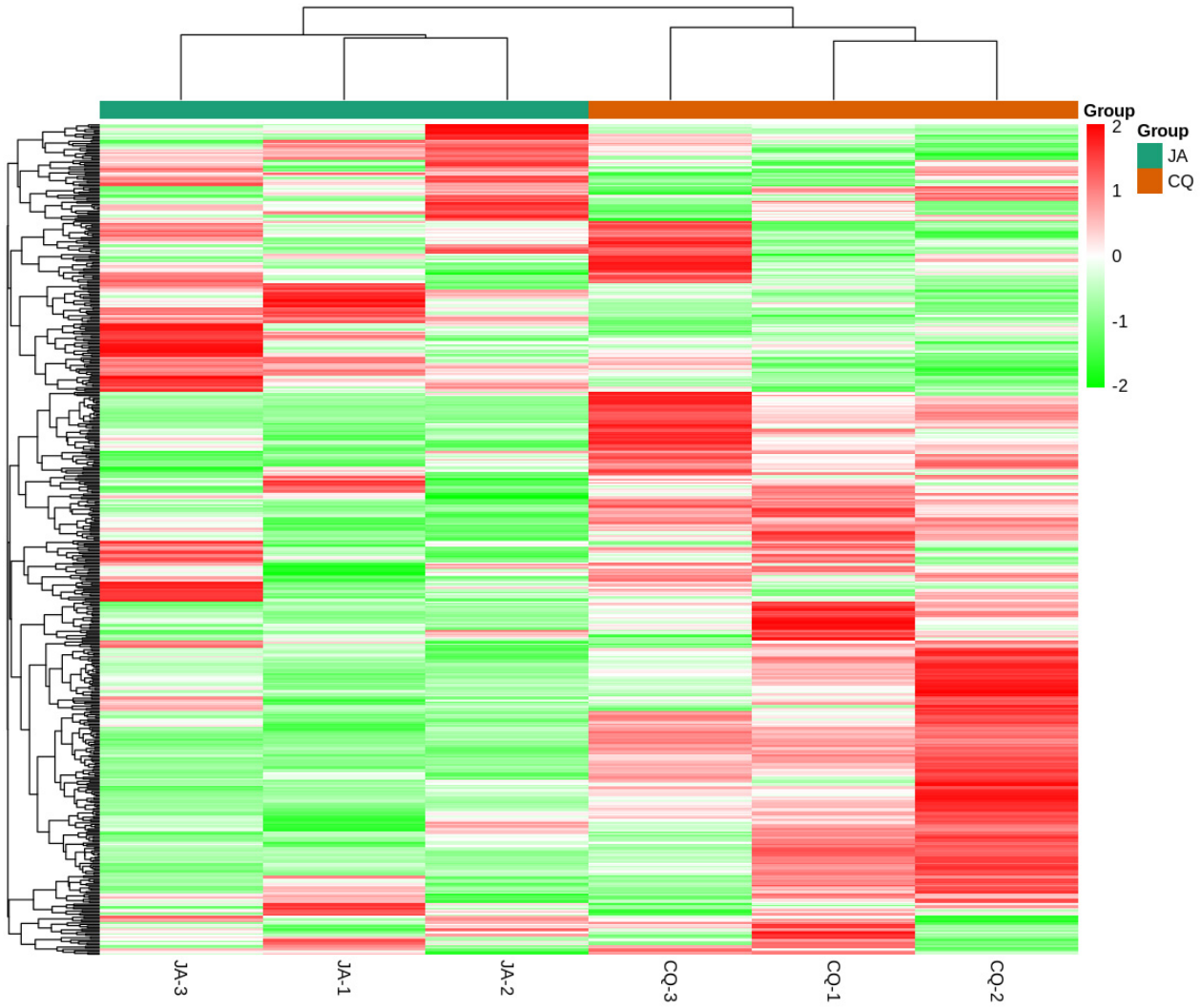

Fig. 2. Hierarchical clustering analysis (HCA) of metabolites identified in Chinese cabbage Gailiangqingza No.3 planted in Jiaozhou (JA) and Changqing (CQ). Red indicates high abundance; green indicates low abundance.

and derivatives, 40 organic acids, 28 flavonoids, 13 lignans and coumarins, three tannins, two terpenoids, and 100 other metabolites were detected, both in JA and CQ. In comparison, only two metabolites, including one amino acid and derivative (S-(methyl)glutathione) and one nucleotide and derivative (nicotinic acid adenine dinucleotide), were unique in JA (Table 2, **Supplementary Table 2**).

Lipids, phenolic acids, amino acids, and derivatives were the most abundant in Chinese cabbage. Among the 96 lipids, the number of free fatty acids was the most abundant (43), followed by lysophosphatidylcholine (LPC) (22), lysophosphatidyl ethanolamine (LPE) (18), glycerol ester (10), sphingolipids (2), and phosphatidylcholine (PC) (1). For the 100 other metabolites (class I), saccharides and alcohols were the most abundant (47), followed by glucosinolates (33), vitamins (13), and other metabolites (class II) (7) (Table 2, **Supplementary Table 2**).

The quantities of amino acids and derivatives, alkaloids, nucleotides and derivatives, organic acids, flavonoids, lignans and coumarins, tannins, and terpenoids had no significant differences between JA and CQ. In contrast, those of lipids and phenolic acids were significantly different ($p < 0.01$ and $p < 0.05$, respectively). In addition, we assumed that the different amount of amino acid derivatives and nucleotides and derivatives between JZ and CQ correlates to soil environmental factors namely N, P, and K content. In pepino (*Solanum muricatum*), N and organic matter increased the content of amino acids and derivatives, alkaloids, and nucleotides and derivatives [27]. Similarly, our finding demonstrates that higher N content in JA (Table 1) affects total amino acids and derivatives as well as nucleotides and derivatives in JA (Table 2). Furthermore, the quantities of lipids and phenolic acids in JA were lower than in CQ. In addition, of the four class II classes of the other metabolites, the quantity of glucosinolates was significantly higher in CQ than in JA ($p < 0.01$). The other three class II classes had no significant differences between CQ and JA (Fig. 3a).

Table 2. Classification of metabolites and differential metabolites identified in Jiaozhou (JA) and Jinan (CQ).

Class I	Number of metabolites		Class II	Number of metabolites	
	JA	CQ		JA	CQ
Lipids	96	96	Free fatty acids	43	43
			Glycerol ester	10	10
			Lysophosphatidylcholine (LPC)	22	22
			Lysophosphatidyl ethanolamine (LPE)	18	18
			Phosphatidylcholine (PC)	1	1
			Sphingolipids	2	2
Phenolic acids	82	82	Phenolic acids	82	82
Amino acids and derivatives	81	80	Amino acids and derivatives	81	80
Alkaloids	45	45	Alkaloids	19	19
			Phenolamine	12	12
			Plumerane	12	12
			Quinoline alkaloids	2	2
Nucleotides and derivatives	41	40	Nucleotides and derivatives	41	40
Organic acids	40	40	Organic acids	40	40
Flavonoids	28	28	Chalcones	1	1
			Dihydroflavone	1	1
			Flavanols	1	1
			Flavonoid	8	8
			Flavonoid carbonoside	1	1
			Flavonols	16	16
Lignans and coumarins	13	13	Coumarins	10	10
			Lignans	3	3
Tannins	3	3	Tannins	3	3
Terpenoids	2	2	Terpenoids	2	2
Others	100	100	Saccharides and alcohols	47	47
			Glucosinolates	33	33
			Vitamins	13	13
			Others	7	7
Total	531	529	Total	531	529

3.2.2 Nutritional Quality-Related Metabolites of Chinese Cabbage

Proteins, carbohydrates, and fats are the human body's three most important nutrients. Amino acids and derivatives, saccharides and alcohols, and lipids are the main metabolites for synthesizing proteins, carbohydrates, and fats. In the study, many kinds of amino acids and derivatives, saccharides, alcohols, and lipids were identified (Table 1, **Supplementary Table 1**, Fig. 2). It is worth mentioning that Chinese cabbage contains all eight essential amino acids required by the human body. L-phenylalanine, L- lysine, L-tryptophan, L-methionine, L-threonine, L-isoleucine, L-leucine, and L-valine are the eight essential amino acids required for normal health and growth in many vertebrates. They are either not manufactured in the body or in insufficient quantities, so they are usually supplied by dietary protein. Among the 81 amino acids and derivatives detected in the study, 32 were the eight essential amino acids or their derivatives, including nine derivatives of L-phenylalanine, L-lysine and its two derivatives, L-tryptophan with one derivative, L-methionine and its three derivatives, L-threonine and L-isoleucine with two derivatives, L-leucine and eight derivatives, and L-valine (Table 3, **Supplementary Table 2**).

Vitamins are types of essential micronutrients for human life. They are important active substances for human health. Generally, they cannot be produced by the human body but must be obtained from the diet. In the study, a total of 13 types of vitamins were detected in Chinese cabbage, including 10 B vitamins (thiamine (vitamin B1), nicotinic acid (vitamin B3), nicotinate D-ribonucleoside (a derivative of vitamin B3), N-(beta-D-glucosyl)nicotinate (a derivative of vitamin B3), nicotinamide (a member of vitamin B), D-pantothenic acid (vitamin B5), pyridoxal (one of the components of vitamin B6), 4-pyridoxic acid-O-glucoside (a derivative of vitamin B6), and orotic acid (vitamin B13), two types of vitamin C (L-ascorbic acid (vitamin C) and dehydroascorbic acid), delta-tocopherol (vitamin E), and menatetrenone (vitamin K2) (Fig. 3b). The results indicate that Chinese cabbage is rich in B vitamins (Fig. 3b).

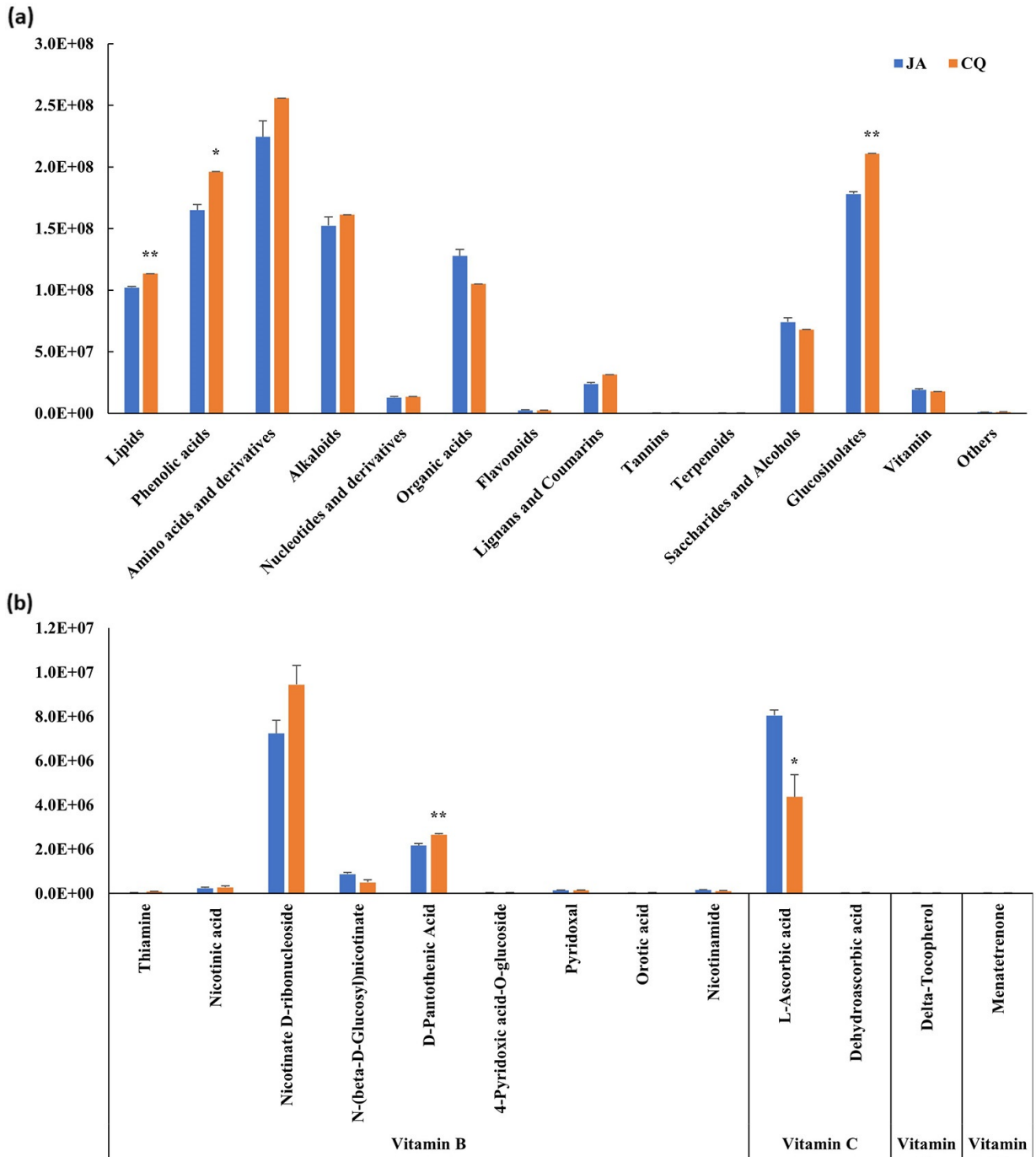

Fig. 3. Quantitation of metabolites detected in Chinese cabbage. (a) Classes and quantitation of metabolites detected in Chinese cabbage. (b) Types and quantities of vitamins detected in Chinese cabbage. *: Difference significance at $p < 0.05$ level according to independent T-test between JA and CQ; **: Difference significance at $p < 0.01$ level according to independent T-test between JA and CQ.

3.3 Taste-Related Metabolites of Chinese Cabbage

Carbohydrates, organic acids, amino acids, and phenolics play important roles in taste determination [28]. In the study, many kinds of carbohydrates, organic acids, amino acids, and phenolics were detected in Chinese cabbage (Fig. 3a). These metabolites may play important roles in the specific taste of Chinese cabbage.

3.4 Chinese Cabbage Metabolites Related to Human Diseases Resistance

Chinese cabbage has been reported to have good medicinal value for many diseases, such as cancer, tumors, inflammation, pathogen infection, diabetes, and so on [29–31]. However, there is no comprehensive identification of the metabolites related to human disease in Chinese cab-

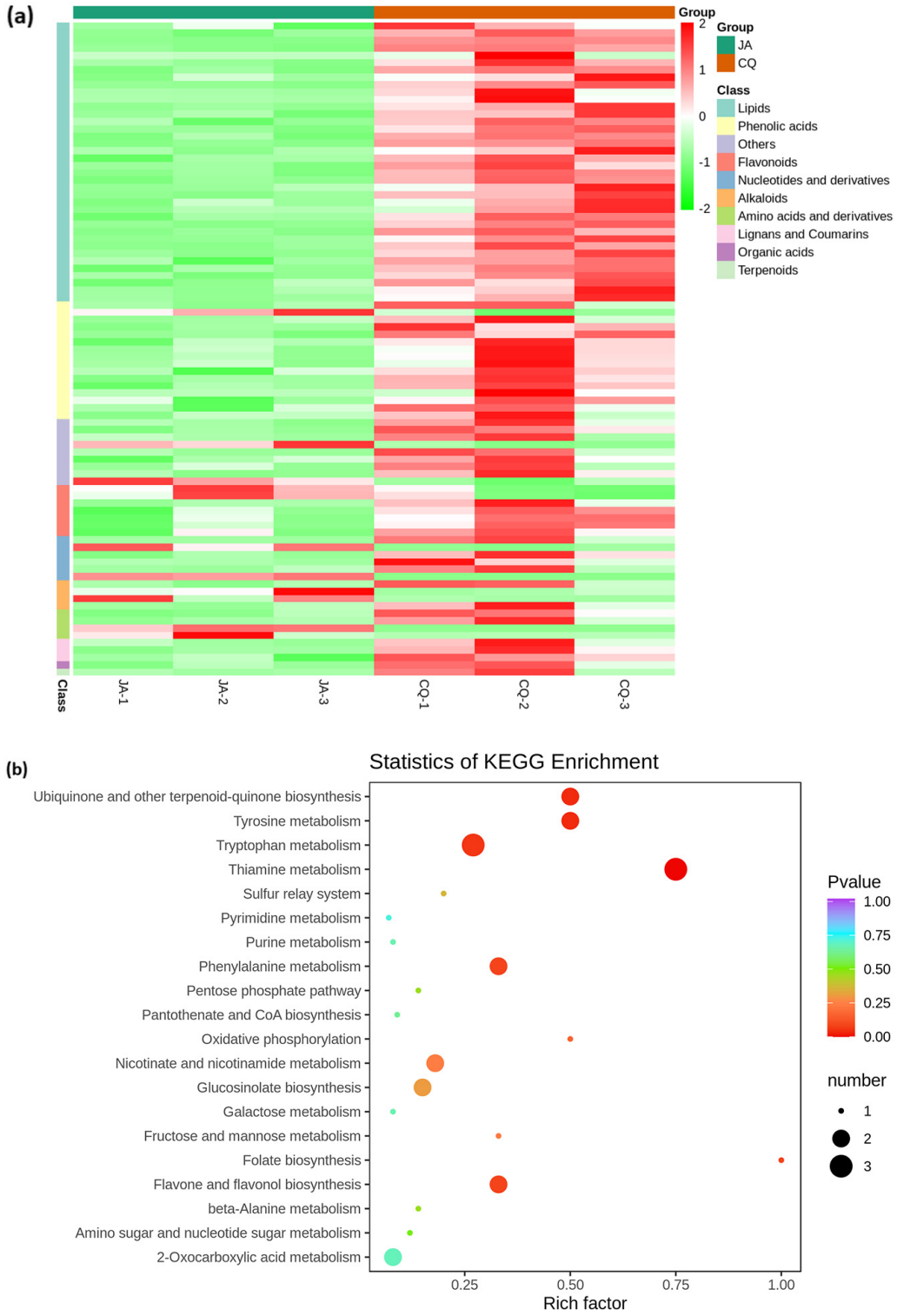

Fig. 4. HCA and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of differentially altered metabolites (DAMs) between JA and CQ. (a) Heatmap of the DAMs detected in all samples. Red indicates high abundance; green indicates low abundance. (b) KEGG enrichment analyses of DAMs between JA and CQ.

bage [32–34]. Therefore, all the metabolites detected in the study were used to perform a query in the TCMSP database to obtain those related to human disease resistance [35]. A total of 108 out of the 531 metabolites were found to be the chemical compositions of Traditional Chinese Medicine, and 79 were found to correspond to at least one disease (**Supplementary Table 3**). The 79 metabolites contained 17 phenolic acids, 13 amino acids and deriva-

Table 3. The relative contents of the eight essential amino acids and their derivatives are identified in Chinese cabbage.

Class	Compounds	JA (Relative contents)		CQ (Relative contents)	
		Mean	SE	Mean	Standard Error (SE)
L-isoleucine	L-isoleucine	5,319,500	386,744	4,914,400	164,068
	L-glycyl-L-isoleucine	567,667	17,138	753,710	101,455
	L-seryl-L-isoleucine	19,007	2389	18,302	882
L-leucine	L-leucine	4,957,000	341,678	4,488,867	298,083
	N-glycyl-L-leucine	1,710,567	90,875	2,106,467	239,557
	Cycloleucine	603,487	37,647	670,747	29,537
	L-alanyl-L-leucine	387,350	41,882	442,963	26,873
	N-acetyl-L-leucine	212,103	38,460	222,310	42,842
	L-prolyl-L-leucine	145,033	18,073	136,723	12,124
	L-leucyl-L-leucine	56,075	1509	67,638	11,646
	L-valyl-L-leucine	25,807	1070	41,847	4464
	Cyclo(Pro-Leu)	3171	238	3051	246
L-lysine	L-lysine	11,224,333	445,047	16,469,000	1,501,237
	N6-acetyl-L-lysine	473,807	65,852	729,123	62,295
	L-lysine-butanoic acid	354,283	87,539	605,603	4648
L-methionine	L-methionine	8,230,400	973,273	8,619,133	797,862
	L-homomethionine	7,884,400	1,127,604	12,804,333	708,177
	L-methionine sulfoxide	2,308,367	126,040	2,597,800	282,279
	L-dihomomethionine	1,755,200	251,214	4,712,200	732,297
L-phenylalanine	L-prolyl-L-phenylalanine	250,057	18,572	215,220	10,458
	L-glycyl-L-phenylalanine	113,378	17,295	114,880	7502
	L-valyl-L-phenylalanine	87,726	5923	95,224	1685
	L-alanyl-L-phenylalanine	59,517	2097	66,127	3569
	L-aspartyl-L-phenylalanine	21,487	1857	19,828	592
	L-leucyl-L-phenylalanine	17,334	2414	21,193	1619
	N-acetyl-L-phenylalanine	4294	663	7069	573
	L-phenylalanyl-L-phenylalanine	3186	617	3543	406
	Cyclo(Phe-Glu)	3144	1347	2892	302
L-threonine	L-threonine	1,786,667	31,645	2,762,533	181,826
L-tryptophan	L-tryptophan	9,834,933	1,475,218	8,682,033	405,642
	5-hydroxy-L-tryptophan	7378	627	19,534	4897
L-valine	L-valine	15,274,667	997,273	19,219,667	276,997

tives, nine lipids, eight alkaloids, seven flavonoids, five nucleotides and derivatives, five organic acids, five vitamins, four lignans and coumarins, two glucosinolates, one saccharide and alcohol, one terpenoid, and one other metabolite (**Supplementary Table 3**). It was validated that Chinese cabbage is a beneficial vegetable for daily health promotion.

3.5 Differentially Altered Metabolites (DAMs) between JA and CQ

It was reported that the production area had obvious effects on the quality of Chinese cabbage because of the different soil and climatic conditions [36]. However, it is still unclear whether and how production areas affect the metabolite profiling of Chinese cabbage. Jiaozhou is one of the most famous main producing areas for Chinese cabbage. Jiaozhou Chinese cabbage has unique taste and flavor qualities [9]. To study the Jiaozhou-specific metabolite profiling of Chinese cabbage, we analyzed the metabolite differences between JA and CQ, and a total of 89 differential metabolites were identified, which consisted of 38 lipids, 16 phenolic acids, seven flavonoids, six nucleotides and derivatives, four amino acids and derivatives, four saccharides and alcohols, four alkaloids, three glucosinolates, three lignans and coumarins, one organic acid, one vitamin, one terpenoid, and one other metabolite (Fig. 4a, **Supplementary Table 4**). Of the 89 differential metabolites, 11 were significantly higher in JA, whereas 78 were significantly lower compared with CQ (Fig. 4, **Supplementary Table 4**). Nicotinic acid adenine dinucleotide, S-(methyl)glutathione, and p-coumaroyltyramine were the top three differentially altered metabolites with the biggest Log2(fold change) value between contents in JA and CQ (the Log2(fold change) values of JA/CQ were 17.89, 14.34, and 2.51, respectively).

Furthermore, lysoPE 16:1 (2n isomer), caffeoyl(p-hydroxybenzoyl) tartaric acid, and lysoPE 16:1 (2n isomer)

were the top three with the lowest Log2(fold change) value (the Log2(fold change) of JA/CQ was –3.86, –3.52, and –2.94, respectively) (Table 3). The results indicate that the producing areas had obvious effects on the metabolite profiling of Chinese cabbage. Nicotinic acid adenine dinucleotide and S-(methyl)glutathione were not detected in CQ, whereas their relative levels were high in JA (**Supplementary Table 3**). These metabolites may be Jiaozhou-specific metabolites of Chinese cabbage. KEGG pathway enrichment analysis was further performed, and the results showed that the DAMs were enriched into 23 pathways, of which tryptophan metabolism (ko00380) and thiamine metabolism (ko00730) were the most significant enrichment pathways (Fig. 4b).

Fourteen out of the 89 DAMs were found to be chemicals related to Chinese traditional medicine, and they corresponded to at least one human disease. The relative contents of 12 DAMs corresponding to human disease were higher in CQ than in JA, whereas only two had lower contents in CQ than in JA (**Supplementary Table 4**). This indicates that Chinese cabbage planted in CQ may have better health effects for some human diseases.

4. Conclusions

In this study, we performed widely targeted metabolite analyses based on an UPLC-MS/MS approach to study the metabolite profiling of the same Chinese cabbage variety Gailiangqingza No.3 planted in two different areas, Jiaozhou and Jinan. A total of 531 metabolites were detected, of which 529 were present in both areas, of which two may be Jiaozhou Chinese cabbage-specific metabolites. The metabolite profiling analysis showed that Chinese cabbage is rich in nutritious substances such as lipids, phenolic acids, amino acids and derivatives, nucleotides and derivatives, alcohols and vitamins, organic acids, flavonoids, glucosinolates, and saccharides. Based on the TCMSP database, many metabolites were also found to be Chinese traditional medicine's chemical compositions, corresponding to at least one disease. The results indicate that Chinese cabbage may have medicinal value in addition to its nutritional value. According to comparative metabolomics analysis, the majority of the 89 DAMs showed higher levels in Chinese cabbage planted in Shuangquan, Jinan, than in Anjiagou, Jiaozhou. Tryptophan metabolism and thiamine metabolism were the two significant enrichment pathways of the 23 significantly enriched KEGG pathways. This study provides valuable information on the metabolite profiles and metabolite variations of Chinese cabbage and will be useful for functional Chinese cabbage cultivation.

Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

Author Contributions

JL, MQ, YZ and JG designed the research study. JL and MQ performed the research. FNR, FW, CL, DZ and HL provided help and advices. JL, MQ and FNR wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The plant material of Chinese cabbage (Gailiangqingza No. 3) in this study was a variety of Jiaozhou cabbage, collected from Jiaozhou area, Shandong, China.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2812345.

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