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Phytonutrients and Metabolism Changes in Topped Radish Root and Its Detached Leaves during 1 °C Cold Postharvest Storage

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Abstract: Glucosinolates, lipid-soluble vitamins E and K contents, primary metabolites and plant hormones were analyzed from topped radish root and detached leaf during storage at 1 °C. The topped root was analyzed at 0, 5, 15, 30, and 90 days after storage while the detached leaf was analyzed at 0, 5, 15, 30, and 45 days in an airtight storage atmosphere environment. The results showed that aliphatic glucosinolates were gradually decreased in leaf but not in root. There was a highly significant correlation between tryptophan and 4-methoxyindoleglucobrassicin in both tissues (r = 0.922, n = 10). There was no significant difference in vitamins E and K in leaf and root during storage. Plant hormones partially explained the significantly changed metabolites by tissue and time, which were identified during cold storage. Phenylalanine, lysine, tryptophan, and myo-inositol were the most important biomarkers that explained the difference in leaf and root tissue during cold storage. The most different metabolism between leaf and root tissue was starch and sucrose metabolism. Therefore, different postharvest technology or regimes should be applied to these tissues.

Keywords: radish; cold storage; phytonutrients; glucosinolate

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1. Introduction

Radish (*Raphanus sativus* L.) is an edible root vegetable, although radish leaf is consumed in few Asian countries [1]. In South Korea, radish leaf is called "mucheong" and is a widely consumed food [2]. In China, radish leaf is also widely consumed in soup and pickles. Recent publication has reported that ethyl acetate extract of radish leaf had a significant effect on blood pressure regulation in rats [3]. Due to the cold tolerance, radish can be grown until early winter. Radish root can be stored up to 6 months at cold storage 0–3 °C [4]. However, phytonutrients and primary metabolite changes of radish root and leaf during cold storage have not been intensively reported despite of few reports on radish leaf or root itself [2,5]. Radish leaf may contain beneficial phytonutrients including glucosinolates and vitamins E and K, similar to kale or collards [6], but there is very little information on such phytonutrients during postharvest storage [4].

Radish root has its own sharp and pungent flavor from the hydrolysis of glucosinolates by the endogenous myrosinase enzyme. Glucosinolates are sulfur-containing plant secondary metabolites that are exclusively present in *Brassica* crops. Radish has no or

very little epithiospecifier protein (ESP) activity, resulting in conversion from glucosinolates to isothiocyanates, which attribute to the pungent flavor [7–9]. Since the hydrolysis products from radish glucosinolates have health-promoting and antibacterial effects, there were many reports on these bioactivities [10]. However, these hydrolysis product concentration changes during cold storage have not been comprehensively investigated with other metabolites.

Vitamin E has been reported to be associated with coronary heart disease, cancer, eye disorders, and cognitive decline [11]. Among different vitamin E in vegetables, α-tocopherol is considered to possess the highest vitamin E activity in many green vegetables [12,13]. However, to our knowledge, this information has not yet been reported for radish leaf. It has been reported that vitamin K is involved in blood coagulation, bone health, and reducing the risk of cardiovascular diseases [14,15]. One study had reported that dietary intake of vitamin K is inversely associated with mortality risk of cardiovascular, cancer, or all-cause mortality in a Mediterranean cohort [16]. Brassica crops are an excellent source of vitamin K [6]. Vitamin K concentrations in 1 g freeze-dried raw collard or kale ranged from 38 to 78 μg [6], which is higher than the adequate intake for an adult male (120 μg) and female (90 μg) [17]. Although leafy brassica vegetables are the major source of vitamin K in the daily diet, the content of vitamin K in radish leaf is not yet reported.

Plant hormones including jasmonic acid (JA), abscisic acid (ABA), and ethylene regulate metabolism of plant under stress conditions. Preharvest stress condition such as insect damage, methyl jasmonate treatment, or ethylene can change on glucosinolates and their hydrolysis products during postharvest storage [18,19]. Drought stress during cultivation affects the glucosinolate metabolism of plants, resulting in overall changes in metabolomes and health-promoting effect [20–22]. Therefore, dehydration during postharvest storage also affects the metabolomes and quality of crop [23]. However, comprehensive postharvest research on plant hormones, phytonutrients, and overall metabolite changes of radish plants have not been evaluated.

Mass spectrometry-based metabolomics are powerful analytical techniques used for metabolic profiling for plant physiology [23–26] or biomarker selection for the discrimination of production methods and consumer-preference-related metabolites [25,27,28]. It has long been utilized in various fields of biological science and enables the identification and quantification of a broad range of metabolites including primary- or secondary metabolites or plant hormones [29] simultaneously. Therefore, advantages in theses technology have led to the development of high-throughput metabolite profiling for physiological change to stresses [30] or biomarker identification for authenticity [31]. Therefore, the goal of this study was to provide a more comprehensive phytochemical and physiological comparison between radish leaf and root during cold postharvest storage. We also measured plant hormones and primary metabolites changes during 1 °C postharvest storage.

2. Materials and Methods

2.1. Plant Materials and Postharvest Storage

'Bac Cheong Moo' Radish seeds were purchased from Holt Garden Center (Montclair, CA, USA). After sowing (25 July 2017) to 36-cell plug trays filled with commercial potting mix (Sunshine LC1 professional, Sun Gro Horticulture, Agawam, MA, USA), seedlings were grown in a greenhouse (24/18 °C as day/night temperature regime). Supplemental high-pressure sodium lighting (600 W HS200 deep reflector; Hortilux, Pijnacker, the Netherlands) was used when the light intensity was below 50 W m^{-2}. Thirty days after sowing (25 August 2017), seedlings were transplanted to a 7 L pot and grown until the mature stage (harvested on 11 October 2017). After transplanting, 20-20-20 Jack's Professional Water Soluble Fertilizer (JR Peters Inc., Allentown, PA, USA) was applied twice a week. Each leaf and root was cut and separately harvested as a biological replication. Before storage, radish leaf and root were washed with 200 mg/L sodium hypochlorite [32], dried at room temperature to remove excessive water on the surface for 2 h, and stored at 1 °C in a walk-in refrigerator (humidity was 50%). Individual radish roots were stored in a zipper

bag (3.7 L, low-density polyethylene with 50 micron) and sampled at 0, 5, 15, 30, and 90 d of postharvest storage while the radish leaves was stored in a 50 L plastic bag (low-density polyethylene with 50 micron) and sampled at 0, 5, 15, 30, and 45 d during airtight atmosphere postharvest storage (void volume was removed as much as possible to ensure identical conditions. Averaged fresh weight of radish root and 4 matures leaves were 566 g (92 g as SD) and 270 g (41 g as SD). After postharvest storage, these samples were freeze-dried, ground, and stored at −20 °C for phytonutrient analyses.

2.2. Glucosinolates Quantification

Desulfatased glucosinolates were quantified according to the method of Ku et al. [33]. Freeze-dried radish powder (0.2 g) was extracted with 4.0 mL of 70% methanol at 95 °C for 10 min. Tubes were cooled on ice, and an internal standard (0.907 mM glucosinalbin, isolated from *Sinapis alba*) was added. Then, tubes were vortexed and centrifuged at 12,000× *g* for 2 min at room temperature. After the supernatants were collected, pellets were re-extracted as described above. Then, 1 mL extract was collected and a mixture of 1 M lead acetate and 1 M barium acetate (1:1, *v*/*v*) (0.15 mL) was added to the pooled extract for protein precipitation. After centrifugation at 12,000× *g* for 2 min, 1 mL of supernatant was loaded onto a column containing DEAE Sephadex A-25 resin pre-charged. After ion exchange resin purification, 3 mL of 0.02 M pyridine acetate was applied followed by washing with 3 mL of distilled water. Then, samples were incubated with 0.5 mL of sulfatase solution 14 h at 21 °C. Desulfatased glucosinolates were eluted with distilled water (1.5 mL) and filtered with a 0.22 µm syringe filter (nylon). Filtered samples were analyzed with a Nexera-i LC 2040C ultra-high performance liquid chromatograph (UHPLC, Shimadzu, Kyoto, Japan) equipped with a photodiode array detector and Kromasil RP-C18 column (250 mm × 4.6 mm). The mobile phase condition and UHPLC operation condition were identical to the previous method [33].

2.3. Glucosinolate Hydrolysis Product

Hydrolytic products of glucosinolates were quantified using a GC-MS following the method of [26] with slight modification [34]. Lyophilized radish powder (75 mg) was suspended in 1.5 mL distilled water in 2 mL centrifuge tubes. After vortexing (5 min) and vertical mixing (up-and-down), the tubes were centrifuged at 12,000× *g* for 2 min. A measure of 0.1 mL of supernatant was transferred to a 1.5 mL PTFE tube (Savillex Corporation, Eden Prairie, MN, USA) followed by 0.4 mL of water and 0.5 mL of methylene chloride containing phenyl isothiocyanate (8 µg mL^{-1}) as an internal standard [20]. Samples were incubated at room temperature for 24 h to hydrolyze glucosinolate by endogenous myrosinase in the absence of light [35]. After allowing the enzyme reaction, sample tubes were centrifuged (12,000× *g* for 2 min), and the lower methylene chloride layer was transferred to a GC vial with an insert. A Trace 1310 GC (Thermo Fisher Scientific, Waltham, MA, USA) coupled to a ISQ QD MS detector system (Thermo Fisher Scientific) and an Triplus RSH autosampler (Thermo Fisher Scientific) was used for the quantification of glucosinolate hydrolytic products. A TG-SQC column (15 m × 0.25 mm × 0.25 µm, Thermo Fisher Scientific) was used to determine glucosinolate hydrolytic products. A 1 µL methylene chloride extract was injected into the GC-MS with the split mode (1:1). The initial temperature of the GC oven was 35 °C, which was held for 1 min. The oven temperature was increased to 310 °C at a rate of 40 °C min^{-1} and held for 5 min. The inlet and ion source temperatures were set at 270 and 300 °C, respectively. The electron ionization mass scan range was 40–450 *m*/*z*. The flow rate of the helium carrier gas was set at 1.2 mL min^{-1}. Authentic standard compounds and the National Institute of Standards and Technology (NIST) library were used for identification [26,36,37]. Individual glucosinolate hydrolytic products were quantified using phenyl isothiocyanate [19].

2.4. Vitamins E and K Analyses

Vitamins E and K were quantitated from 0.3 g of radish tissue (freeze-dried) according to the method of Xiao et al. [38] with some modifications [6]. Vitamins E and K were quantified using UHPLC with the published method [6].

2.5. Primary Metabolite Extraction and Analysis

Primary metabolites analysis was conducted using previous protocols [39] with modifications [40]. Freeze-dried samples of 50 mg each were extracted with 1.4 mL of methanol at 75 °C after adding 80 µL of ribitol as internal standard (10 mg mL^{-1}) in 2 mL microcentrifuge tubes. Sample tubes were centrifuged at 15,000× *g* for 3 min, and 0.7 mL supernatants were transferred to new 2 mL microcentrifuge tubes. To purify polar compounds, 0.375 mL of cold chloroform (stored at −20 °C) and 0.7 mL cold water (4 °C) were added for liquid-to-liquid extraction. After mixing with a vortex, the tubes were centrifuged (15,000× *g* for 3 min). Measures of 50 µL supernatant from the sample tubes were transferred to 1.5 mL microcentrifuge tubes. This supernatant was dried using Vacufuge concentrator (Eppendorf, Thermo Fisher Scientific, Waltham, MA, USA). After drying, dried extracts were derivatized with 50 µL methoxyamine hydrochloride (40 mg mL^{-1} in pyridine) for 90 min at 37 °C. Then, 70 µL MSTFA + 1% TMCS was additionally added to the tubes and incubated for derivatization at 37 °C for 30 min. Polar metabolites were analyzed using a GC-MS (Trace 1310 GC, Thermo Fisher Scientific, Waltham, MA, USA) connected to a ISQ QD mass spectrometer (MS) detector system (Thermo Fisher Scientific) and an Triplus RSH autosampler (Thermo Fisher Scientific). An Rxi-5Sil MS column (Restek, Bellefonte, PA, USA; 30 m × 0.25 mm × 0.25 µm with 10 m Integra-Guard Column) was used to measure polar metabolites. The initial temperature started at 80 °C and was held for 2 min; the oven temperature was increased to 330 °C with 15 °C min^{-1} and held for 5 min. The injector and detector temperatures were 250 °C and 250 °C, respectively. A measure of 1 µL was injected with a split ratio of 200:1. The flow rate of helium carrier gas was 1.2 mL min^{-1}. The MS was operated in electron impact mode at 70.0 eV ionization energy with a m/z 40–500 scan range. The metabolite results were normalized to ribitol (for sample-wise normalization) in online platform MetaboAnalyst and further statistical analysis was conducted after auto-scaling for feature-wise (metabolites) normalization. Authentic standard compounds and National Institute of Standards and Technology (NIST) library were used for identification.

2.6. Amino Acid Quantification

EZ:faast free amino acid for the GC-MS kit (Phenomenex, Torrance, CA, USA) was quantitated free amino acid in the sample. A measure of 75 mg of freeze-dried sample was incubated at room temperature in 1.5 mL water overnight to extract free amino acids from the sample. Then, samples were centrifuged at 12,000× *g* for 3 min. Amino acid purification and derivatization were conducted on EZ:faast instruction with recommended GC-MS conditions.

2.7. Plant Hormone Analysis

Freeze-dried sample (50 mg DW.) was extracted in a 2 mL tube with 1 mL of extraction solvent (methanol:isopropanol:glacial acetic acid, 20:79:1, v/v) using ultra sonication with ice. The labeled forms of the compounds d_6-ABA, d_5-JA, and d_4-ACC were added as internal standards. Supernatants were collected after centrifugation (10,000 rpm for 5 min), and the pellets were re-extracted with 0.5 mL of extraction solvent. Two times supernatants of extracts were combined and filtered through a 0.22 µm PTFE filter (Waters, Milford, MA, USA). Samples of 5 µL were injected to UPLC with AB Sciex Qtrap 5500 triple quadrupole ESI-MS/MS. Kromasil C_{-18} column (2.1 × 50 mm, 2.5 µm) was used. Gradient elution was performed with water and 0.05% glacial acetic acid (solvent A) and acetonitrile with 0.05% glacial acetic acid (solvent B) at a constant flow rate (0.6 mL min^{-1}). The optimized

multiple reaction mode MS/MS conditions for the analysis of plant hormones are well introduced in Plant Methods [29].

2.8. Statistical Analysis

All analyses were conducted from 3 biological replications (consisting of 2 samples). Each biological replication for root or leaf (was consisted of) has 2 roots or 4 mature leaves from 2 plants, respectively. Univariate analysis of variance and Tukey's test were performed using Graphpad Prism (San Diego, CA, USA) to determine the significance of the phytonutrient content during cold storage. For heatmap analysis, GC-MS-based primary metabolite data and GC-FID amino acid data were combined and processed using MetaboAnalyst 4.0 [41]. Heatmaps and biomarker selections were performed by partial least-squares discriminant analysis (PLS-DA) in MetaboAnalyst 4.0.

3. Results

3.1. Glucosinolates

Glucosinolate concentrations in different storage periods of radish are shown in Figure 1A. A total of 14 glucosinolates were detected in radish with different storage times, including 8 aliphatic (glucoraphasatin, glucoraphenin, glucoraphanin, glucoiberin, progoitrin, gluconapin, sinigrin and glucoerucin), 5 indole (glucobrassicin, 4-methoxy glucobrassicin, 1-hydroxy-glucobrassicin, neoglucobrassicin, and 4-hydroxy glucobrassicin), and 1 aromatic (gluconasturtiin) glucosinolates. Glucoraphasatin and glucoraphenin are the major aliphatic glucosinolates in radish leaf and root, which was in agreement with the study of [42]. The total aliphatic glucosinolates gradually decreased in leaf (from 15.2 μmole g^{-1} DW at 0 day to 6.5 μmole g^{-1} DW at 45 days after storage). However, the total aliphatic glucosinolates of root was decreased by about 36% during the first 5 days of cold storage (Figure 1B). Glucoraphasatin, a glucosinolate with a four-carbon aliphatic side chain, represented 57.5% and 75.9% of the total glucosinolate content in leaf and root, respectively. The radish root had higher glucoraphasatin than the leaf. These results were consistent with the reports of [7,43], which showed that glucoraphasatin accounted for approximately 74% of the total glucosinolates. During the storage, the glucoraphasatin concentration gradually decreased, resulting in a reduction in the total aliphatic glucosinolates. Compared to day 0, there was a significant difference in glucoraphasatin in radish leaf at day 30 and day 45, whereas there was no difference in the root. The glucoraphasatin ($r = -0.9298$, $p = 0.022$, $n = 5$) and glucoraphanin ($r = -0.9141$, $p = 0.030$, $n = 5$) concentrations significantly correlate with the storage day of the leaf tissue. Indole glucosinolates represented only 17.2% and 2.7% of the total glucosinolates in the leaf and root, and the glucobrassicin content was the highest in radish. There was a significant difference in glucobrassicin between day 0 (3.0 μmole g^{-1} DW) and day 45 (1.6 μmole g^{-1} DW) in radish leaf. Due to the low concentration, the indole glucosinolates of radish root were no different across different storage times. Although 4-methoxy-glucobrassicin is in a low concentration in leaf ($r = 0.9582$, $p = 0.010$, $n = 5$) and root ($r = 0.9183$, $p = 0.030$, $n = 5$), the concentration gradually increased during cold storage and showed significant correlations with the storage day, respectively.

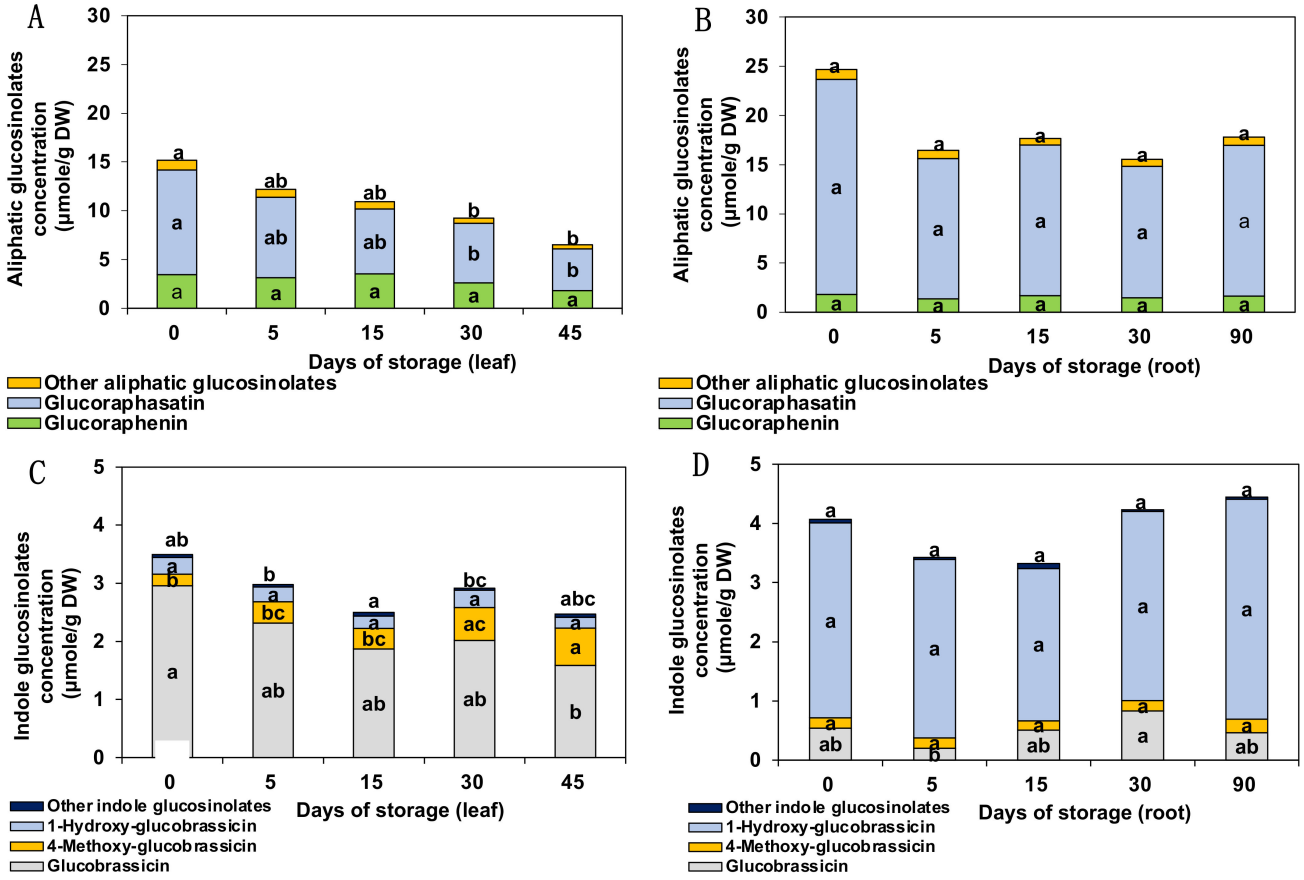

Figure 1. Aliphatic and indole glucosinolate concentration changes in detached leaf (**A**,**C**) and topped radish root (**B**,**D**) during 1 °C cold postharvest storage. Mean is expressed as from triplicates (n = 3). Different letters indicate significant difference across different days of storage within the same compound at $p = 0.05$ by Tukey's test.

3.2. Glucosinolate Hydrolysis Products

Raphasatin and sulforaphene are major glucosinolate hydrolysis products in both the leaf and root of radish (Figure 2), which agree with previous research [44,45]. Raphasatin and sulforaphene concentrations from leaf and root tissue were not significantly different throughout the cold storage, although two compounds were gradually reduced. A previous study reported that the reduction in myrosinase activity and ascorbate content are possible causes of a decreased concentration of raphasatin and sulforaphane [45]. However, in this study, raphasatin and sulforaphane were not significantly decreased. This symptom was also observed in a previous study in which few cultivars did not show a significant loss of raphasatin, sulforaphane, or myrosinase activity after 8 weeks of cold storage [45]. Sulforaphene has significant anti-proliferative potency on human cancer cells via the induction of apoptosis [46]. Raphasatin induced phase II detoxification enzymes from HepG2 cells at a concentration of 10 μM [47]. For the sulforaphane intake, radish leaf can be good option, although the root can be a good source for raphasatin.

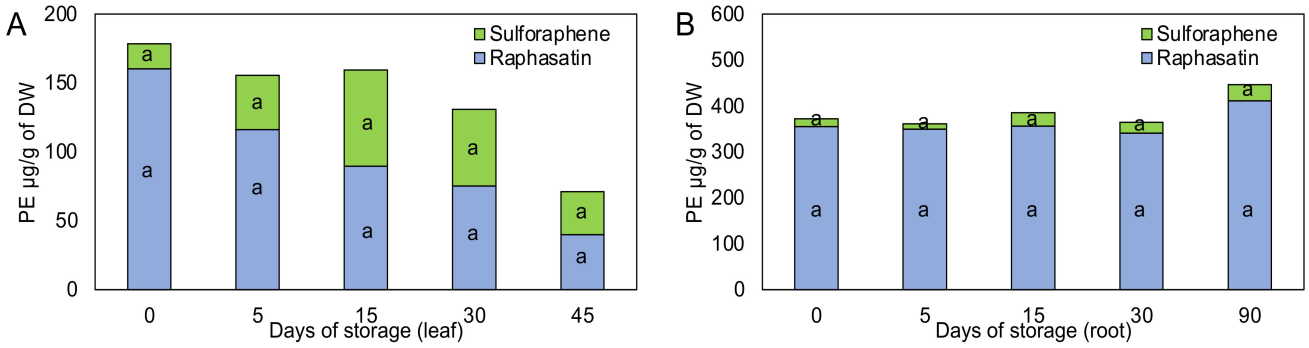

Figure 2. Glucosinolate hydrolysis product concentration (phenyl isothiocyanate equivalent, PE) changes in detached leaf (**A**) and topped radish root (**B**) during 1 °C cold postharvest storage. Mean is expressed as from triplicates (n = 3). Different letters indicate significant difference across different days of storage within the same compound at $p = 0.05$ by Tukey's test.

3.3. Vitamins E and K

α- or γ-tocopherols are the predominant source of vitamin E in plants [12,13]. According to Figure 3, during the storage, the total tocopherol content of the leaf ranged from 27.9 µg g^{-1} DW to 48.5 µg g^{-1} DW with α-tocopherol representing 73.8–93.8% of the total tocopherol. However, the root had a very low tocopherol content, ranging from 0.09 µg g^{-1} DW to 0.17 µg g^{-1} DW during the storage. There was no difference in α-tocopherol but there was some variation in γ-tocopherol. Tocopherols have shown health benefits to cardiovascular disease, inflammation, and other diseases [48,49]. Considering that the recommended intake of vitamin E is 15 mg/day for adults, 100 g of fresh radish leaf used in this study can provide 1.8–3.2% of RDA (water content for 90%).

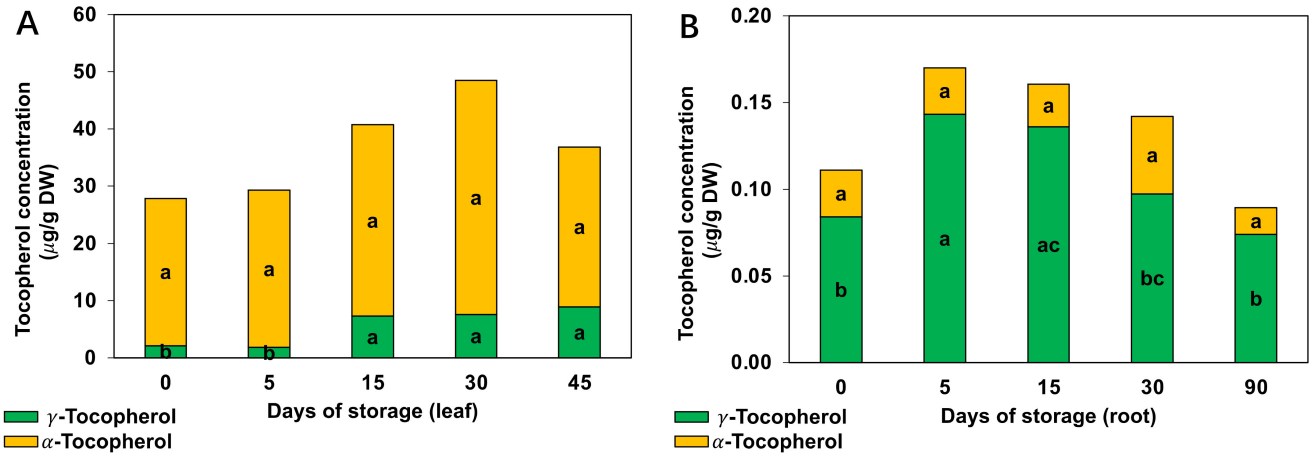

Figure 3. Alpha- and gamma tocopherols concentration changes in detached leaf (**A**) and topped radish root (**B**) during 1 °C cold postharvest storage. Mean is expressed as from triplicates (n = 3). Different letters indicate significant difference across different days of storage within the same compound at $p = 0.05$ by Tukey's test.

Phylloquinone (vitamin K_1) is the most widely found vitamin K in plants and is the predominant form of dietary vitamin K (>90%) [50]. Only leaf samples contain phylloquinone (Figure 4). A previous study reported that phylloquinone is associated with electron carrying during photosynthesis [51], supporting this result. During the storage period, the phylloquinone of the leaf changed from 24.9 µg g^{-1} DW to 30.9 µg g^{-1} DW but there was no significant variation. A previous study reported that 1 g of freeze-dried raw collard or kale contains 38 to 78 µg vitamin K [6], which is higher than radish leaf. However, in this study, 100 g of fresh radish leaf can provide 276.7–343.4% and 207.5–257.5% of RDA for US women and men, respectively, which means radish leaf is an excellent source of

phylloquinone. Note that the adequate intake level of vitamin K in the USA for adult women and men is 90 and 120 µg/day, respectively.

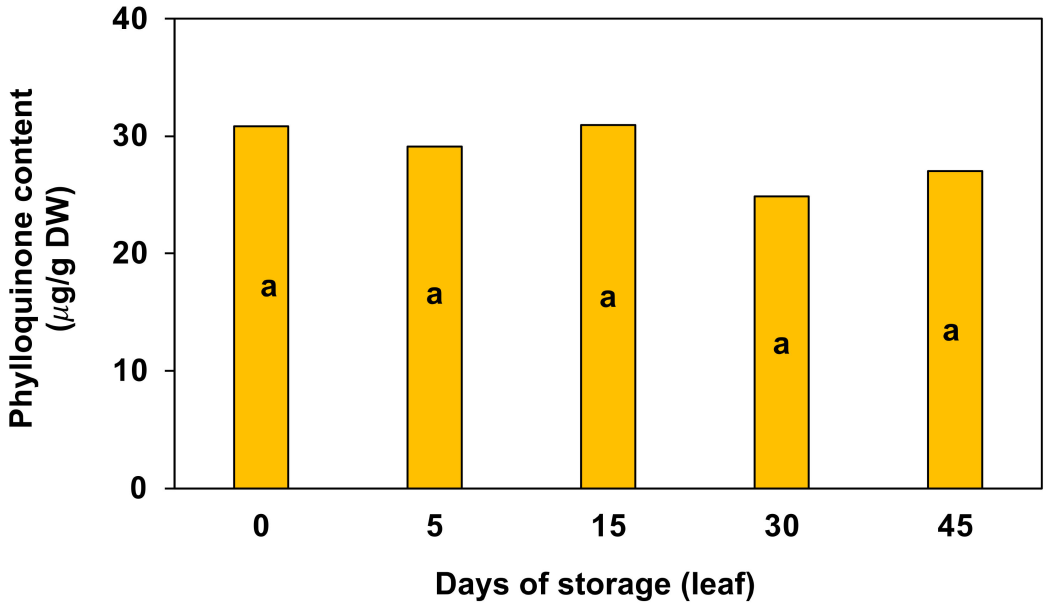

Figure 4. Phylloquinone concentration changes in detached leaf during 1 °C cold postharvest storage. Phylloquinone was only detected in leaf tissue. Mean is expressed as from triplicates (n = 3). Different letters indicate significant difference across different days of storage within the same compound at $p = 0.05$ by Tukey's test.

3.4. Primary Metabolite Extraction and Analysis

The primary metabolite profile of radish leaf and root is shown in Figure 5. The concentrations of most amino acids, sugars, and fatty acids were higher in root tissue, whereas organic acids were higher in leaf tissue. As the storage time increased, amino acids significantly increased whereas fatty acids slightly decreased both in the leaf and root during storage. Meanwhile, the eight most important discriminatory biomarkers were detected between leaf and root (fructose, glucose, myo-Inositol, malic acid, lysine, alanine, phenylalanine, glutamine, and palmitic acid) (VIP value > 1.2). According to the analysis of two-way ANONA, four compounds (phenylalanine, tryptophan, lysine, and *myo*-Inositol) were shown to be significantly different by tissue type (leaf and root) and storage time (Figure 5A). Among these discriminatory biomarkers, four metabolites including phenylalanine, lysine, tryptophan, and myo-inositol were the most important biomarkers that explained difference of leaf and root tissue during cold storage (Figure 5B).

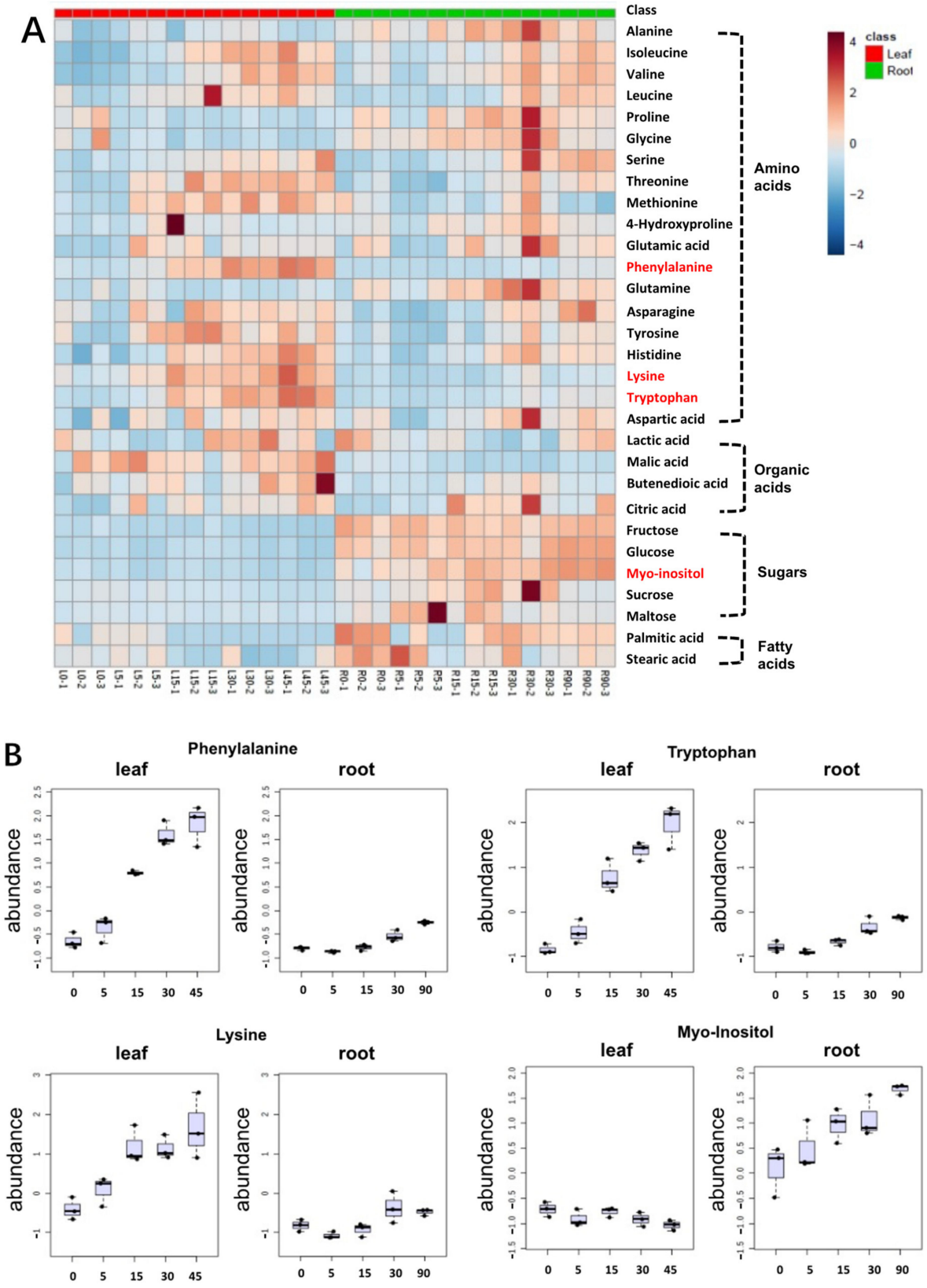

Figure 5. Heatmap of primary metabolites from detached leaf during 1 °C cold postharvest storage (**A**). Compounds in red indicate significantly different biomarkers in in both tissue type (leaf and root) and storage time. The most significantly different primary biomarkers in both tissue type (leaf and root) and storage time (**B**).

3.5. Correlation Analyses between Precursor Amino Acids and Glucosinolate during Storage

There was a significant correlation between tryptophan and 4-methoxyglucobrassicin during storage (r = 0.9292, $p < 0.001$, n = 10, Figure 6). In addition, from the correlation analysis, tryptophan significantly correlates with the storage day (r = 0.6857, $p = 0.029$, n = 10, data was not shown here). Taken together, the increased 4-methoxyglucobrassicin concentrations were synthesized from tryptophan. The free form of tryptophan concentration in leaf tissue was gradually increased during storage, although the small incensement was observed in root tissue (Figure 6). The result of this study suggested that 4-methoxyglucobrassicin biosynthesis is determined by the tryptophan pool during storage. 4-methoxyglucobrassicin has an anti-fungal effect and the biosynthesis pathway is 4-methylation from glucobrassicin [52,53]. This 1-methylation biosynthesis is stimulated by methyl jasmonate during storage in broccoli [19] and is suppressed by ACC in *Arabidopsis* [54]. A previous study also reported that ethylene (1000 ppm) treatment selectively induced the 4-hydroxylation of glucobrassicin in broccoli heads, resulting in ~223% higher 4-hydroxyglucobrassicin than time 0 h samples [55]. The previous results indicate that the methylation site on glucobrassicin is determined by plant hormonal regulation; however, indole glucosinolate conversion during postharvest storage of radish tissues has not been reported yet. In the present study, ethylene or ACC accumulation in leaf tissue during postharvest storage favors the pathway of methoxylation from glucobrassicin to 4-methocyglucobrassicin rather than the formation of neoglucobrassicin. Amino acid methionine was increased during postharvest storage but aliphatic glucosinolate did not increase. This paper suggested the glucobrassicin conversion to methoxy glucosinolate is regulated by plant hormone but as a tissue-specific response.

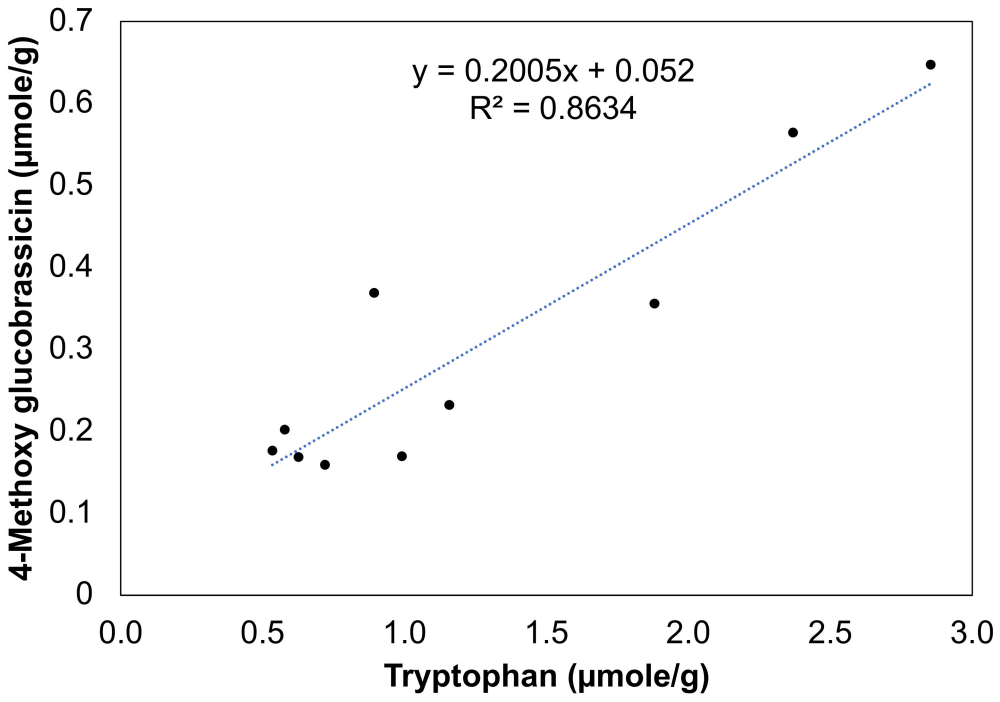

Figure 6. Pearson's correlation (n = 10) between tryptophan and 4-methoxyglucobrassicin in detached leaf and topped radish root during 1 °C cold postharvest storage.

3.6. % Water Loss per Day and Plant Hormone Changes of Radish Root and Leaf during Cold Storage

The percentage of water loss per day from day 0–30 was similar between leaf and root tissue. However, the percentage water loss per day in leaf tissue started to increase at day 30 while the water loss rate slightly decreased in root tissue. The increased percentage water loss may be related with leaf senescence (Figure 7A). The ABA concentration of leaf

tissue was decreased as cold storage started from day 0–5. However, the ABA concentration of leaf tissue started to increase in leaf tissue at day 15. We assume that ABA signaling may be triggered by accumulated water loss during cold storage. At days 30–45, the leaf tissue was severely dried and suffered from senescence. The percentage water loss per day and ABA concentration in leaf tissue were in a similar pattern and numerically higher than root tissue. The ACC concentration was gradually increased in leaf tissue while it was gradually decreased in root tissue at day 30 and stable after 30 days. ACC is the precursor of ethylene. Increased ACC concentration in leaf tissue may be related with drought stress or senescence during storage. We think that the jasmonic acid concentration changed in similar way to ABA.

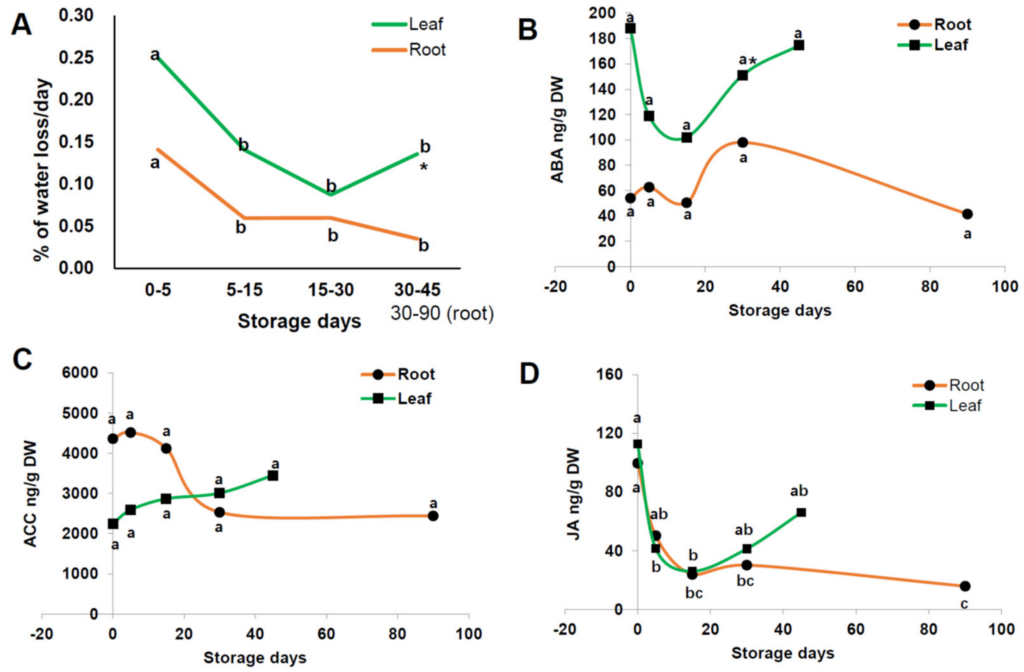

Figure 7. Percentage of water loss per day (**A**), abscisic acid ((**B**), ABA), ethylene precursor ((**C**), ACC), and jasmonic acid ((**D**), JA) changes in detached leaf and topped radish root during 1 °C cold postharvest storage. Different letters indicate significant difference across different days of storage within the same tissue at $p = 0.05$ by Tukey's test. * indicates significant difference between root and leaf tissue within the same storage days or stages.

3.7. Physiological Changes of Radish Root and Leaf during Cold Storage Based on Pathway Analysis

To better understand metabolism changes in two different radish tissues (leaves and root), pathway analysis in MetaboAnalyst was utilized and visualized for radish metabolomic changes during cold storage. Alanine, aspartate, and glutamate ($-\log(p) = 15.8$, impact = 0.64, aspartate, asparagine, alanine, glutamine, and glutamate are major metabolites in this pathway), isoquinoline alkaloid biosynthesis ($-\log(p) = 16.0$, impact = 0.50, tyrosine was the only relevant for this metabolism), starch and sucrose metabolism ($-\log(p) = 25.6$, impact = 0.49, sucrose is the only relevant for this metabolism), glycine, serine, and theronine ($-\log(p) = 3.92$, impact = 0.51, glycine, aspartate, threonine, and tryptophan are major metabolites in this pathway), and arginine and proline ($-\log(p) = 16.5$, impact = 0.19, proline, glutamine, and hydroxyproline are major metabolites in this pathway) were all highly impacted pathways as characterized by both high pathway impact value (0.15) and high $-\log(p)$ value (>10) (Figure 8). The results indicate that two different tissues have significantly different above-mentioned metabolisms during cold storage. The previous study by Jia et al. [56] suggested that the accumulation of aspartate and glutamate compounds was related to drought tolerance in willow species. During cold storage, two different tissues

might have been influenced by different levels of dehydration/drying stress. Arginine and proline metabolism is also known as a drought tolerance mechanism. Proline is a well-known compatible solute to adjust osmotic stress under abiotic stress including salt and drought conditions [57]. From the same pathway analysis using different cold storage days in leaf tissue, starch and sucrose, glycine, serine and threonine, and phenylalanine metabolism and arginine biosynthesis as well as two aforementioned dehydration related metabolisms (alanine and proline; alanine, aspartate, and glutamate metabolism) were significantly different by cold storage days. In a previous study, topped leaves showed significantly higher (four to seven times) water loss compared to root [58], in agreeance with our data. Not only the water loss but active respiration was also observed in leaf tissue since starch and sucrose metabolism is associated with part (glycolysis) of the respiration. The starch and sucrose metabolism differed the most between leaf and root tissue. Leaves usually have many stomas since transpiration mainly occurs in this tissue rather than other tissues. Stoma in leaves and active respiration may explain why the leaves have higher water loss. Water loss of leafy vegetables is closely associated with consumers' purchase decision based on turgidity and freshness of leafy vegetables [59]. Even 3% of weight loss in a leafy vegetable may reduce the consumer's purchase decision [59]. Interestingly, significant starch and sucrose metabolism was not observed in topped root tissue by different cold storage days. This explains the sink–source relationship between leave and root. Based on the different metabolism, it may be a good strategy to divide leaf and root tissue for storage to avoid the effect of high metabolism of the leaf on root tissue, which requires further study. To the best of our knowledge, the current study was the first report of comprehensive physiological and phytochemical research on radish cold storage.

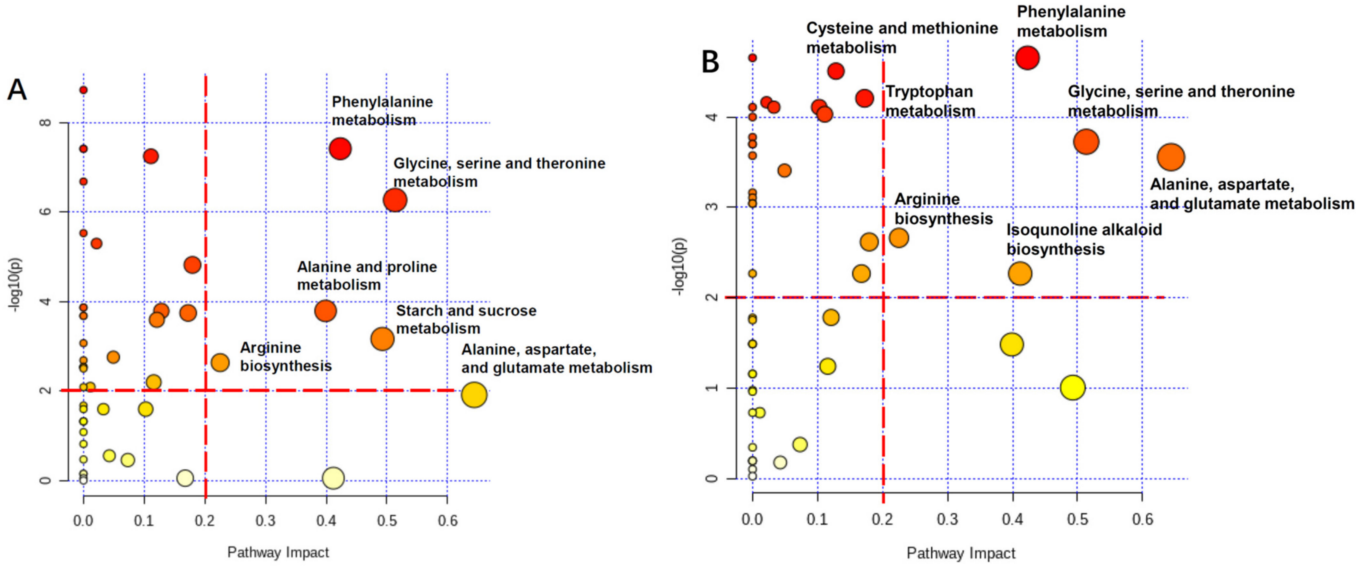

Figure 8. The most significant changes in metabolism pathway by pathway analysis from MetaboAnalyst in detached leaf (**A**) and topped radish root (**B**) during 1 °C cold postharvest storage. Identified metabolism names were displayed based on pathway impact (>0.2) and −log10(*p*) (>2).

4. Conclusions

In the present study, we analyzed the phytonutrients change of radish leaf and root in storage, including glucosinolates, fat-soluble vitamins E (as α- and γ-tocopherols) and K (phylloquinone), and primary metabolite. The aliphatic glucosinolates decreased significantly in leaf but only changed obviously during the first five days in root. Among them, glucoraphasatin represented 57.5% and 75.9% of the total glucosinolate content in leaf and root. According to the analysis of primary metabolites, the eight most important discriminatory biomarkers were detected between leaf and root (fructose, glucose, myo-Inositol, malic acid, lysine, alanine, phenylalanine, glutamine, palmitic acid). Meanwhile, four

compounds (phenylalanine, tryptophan, lysine, and myo-inositol) showed significantly different both in tissue (leaf and root) and storage time. The starch and sucrose metabolisms differed the most between leaf and root tissue. Taken together, radish leaf and root have different phytonutrient profiles and showed different postharvest physiology, suggesting that postharvest technology should consider the tissue-specific physiology.

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