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Stress response and health affecting compounds in Brassicaceae

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Chapter 7

Postharvest storage stability of red radish (*Raphanus sativus* L.) at different temperatures

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Abstract

Radish (*Raphanus sativus* L.) is a well known and commonly consumed vegetable all over the world. Its bioactive or nutritional constituents consist of a wide range of metabolites including, glucosinolates, phenolics, amino acids, organic acids, and sugars. However, many of these metabolites are not stable and easily degraded or modified during storage. In order to investigate the metabolomic changes occurring during post harvest storage, radish samples were subjected to four different storage temperatures (20 °C, 4 °C, -20 °C, and -80 °C) in 28 days storage time course. ¹H nuclear magnetic resonance (NMR) and two-dimensional NMR spectra data were subjected to principal component analysis (PCA) followed by partial least square-discriminant analysis (PLS-DA) to investigate the metabolomic changes.

A profound chemical alteration, both in primary and secondary metabolites was observed. Glucosinolates, phenylpropanoids, amino acids, organic acids, and sugars were found to be the discriminating metabolites for storage effects. At the start of the storage an increase in secondary metabolites (phenylpropanoids, flavonoids and glucosinolates) is observed followed by a decrease in these compounds in later stages. This loss is high at room temperature while lower at cold and freezing temperatures. During the later storage stages, still a high amount of primary metabolites is observed.

Keywords: radish (*Raphanus sativus* L.), storage effect, postharvest storage, ¹H NMR, multivariate data analysis.

1 Introduction

Different post-harvest processing and storage methods are reported to retard the degradation of bioactive compounds and to retain quality attributes in different vegetables.^{89, 413-415} Post-harvest storage (e.g. low temperature storage, freezing, as well as fresh vegetable storage at room temperature), industrial processing (drying, blanching, canning etc.), and different cooking methods play an important role in degradation of *Brassica* bioactive components.⁸⁹ Effect of post harvest storage conditions especially cold storage on plant metabolome has always been a controversial issue. Changes may occur due to pure chemical conversion (fastest at higher temperature) or to residual metabolism of the plants and plant cells after harvesting. The later includes the plant response to wounding, infection, drought and temperature, which is quite complex and unpredictable as one or more responses may be active depending on the conditions. In this study radish presents a special case as roots and green parts remain intact as a whole plant.

It is also possible to prevent loss of nutritional value by using a lower storage temperature,^{35, 416} as it results in decreased rate of metabolism, thus preserving radish quality.⁴¹⁷ But storage at chilling temperature can have both positive and negative effects on vegetables, depending on the commodity and the storage temperature.¹¹ Altered gene expression, e.g. in the response of plants to environmental stimuli, results in qualitative and quantitative changes in the metabolome.³⁵ The constitutive activation of several stress-inducible pathways and different kinetics in the accumulation of several metabolites may represent an advantage to prepare plants to face low temperature stress conditions.⁴¹⁸

The evidence for the importance of health-promoting bioactive compounds present in *Brassica* vegetables has increased in the last few years.⁴¹⁹ Because of the nutritional importance of *Brassica* metabolites, there has been an increasing interest in the evaluation of these compounds in postharvest treatments.⁴²⁰ Radish is one of the most important and worldwide well-known food crop belonging to the Brassicaceae.^{369, 421} Radish is considered to have health benefits due to the presence of sugars, amino acids, organic acids, phenolics, and glucosinolates.^{101, 125, 400, 422} Also it is a well established model system for plant research.^{370, 423, 424} Like many other vegetables, radish can be preserved by storage, pickling, canning or drying² but the chemical composition of its nutritional constituents, especially the phenolics is

easily affected by such pre-harvest agronomic and post-harvest processing and the storage conditions.^{11, 416}

Phenolics, such as phenylpropanoids play an important role in vegetable food quality, such as in appearance, flavour, and antioxidant properties.¹¹ The key enzyme (phenylalanine ammonia lyase, PAL) in the biosynthesis of phenylpropanoids activity is regulated by a diverse array of pre-harvest and post-harvest factors.¹¹ These phenolic compounds can be degraded into other simple phenolics such as vanillic and protocatechuic acid⁴²⁵ along with other degradation products including oxalic, glyoxylic, oxaloacetic, mesoxalic and formic acid.⁴²⁶ In addition to aforementioned degradation products, *Brassica* vegetables include ascorbigens, which are formed as the result of the reaction between ascorbic acid and degradation products of indol-3-yl-methyl-glucosinolates produced in the myrosinase-catalysed degradation.^{427, 428}

The comprehensive quantitative and qualitative analysis of all metabolites within a cell, tissue or organism is a very ambitious goal.³⁵ The components of the metabolome can be viewed as the end products of gene expression and define the biochemical environment of a cell or tissue. Metabolomics provides a broad view of the biochemical status of an organism⁴²⁹ and is largely used to study the phytochemical changes in plants.³⁵ At present no single analytical method can provide information about all the metabolites in plants, since the diversity in plant metabolites is too large; volatility, polarity, solubility, and chromatographic behaviour and detectability differ largely.²⁷ So the selection of the most suitable analytical method is generally a compromise between speed, selectivity and sensitivity.³⁵ Nuclear magnetic spectroscopy (NMR) based metabolomics is becoming increasingly recognized in research and development as a highly reproducible and quantitative method. Although NMR is not as sensitive as some other analytical methods, like HPLC, MS, etc. But it is a non destructive method and can detect a large number and diverse groups of compounds in a single-run.^{35, 37}

A lot of work has been done on the storage stability of vegetables after pre-treatment (e.g. blanching, packing, UV etc.) but there is still need to study the effect of different temperatures with respect to storage time, and without pre-treatment. NMR seems an optimum choice to study the overall major changes in the metabolome during cold storage.

The objective of the present study was to investigate the phytochemical changes in radish metabolome at different time points and temperatures, with particular focus on compounds related to quality of the food plant. To analyze these changes, ¹H NMR and two-dimensional

NMR spectra were used in combination with principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA).

2 Materials and methods

2.1 Preparation of plant material

Red radish (*Raphanus sativus* L.) plants were purchased from local market. Fresh and healthy plants were selected and washed thoroughly with de-ionized water and kept in open air at room temperature for half an hour to remove surface water from plant. The aerial parts (leaves and petioles) and roots were kept intact during storage.

2.2 Storage and sample collection

Plants were stored at four different temperatures [20 °C (room temperature), 4 °C, -20 °C, and -80 °C] in open plastic bags and kept in dark. Samples from each treatment were collected after each 2 h, 4 h, 6 h, 12 h, 24 h, 2 d, 3 d, 7 d, 14 d, 21 d and 28 d. After 3 days plants stored at room temperature started decaying so further sampling was stopped from this condition. Three replicates were used for analysis, with one plant for each replication. The aerial parts (mentioned as Leaves) were separated from roots and both roots and leaves were immediately frozen in liquid nitrogen. Leaves and roots were ground separately in liquid nitrogen and freeze-dried, to obtain a fine powder.

2.3 Extraction of plant material and NMR measurements

Fifty mg of freeze dried material were transferred to a microtube (2 ml) to which 1.5 ml of 50% methanol-*d*₄ in D₂O (KH₂PO₄ buffer, pH 6.0) containing 0.05% TMSP (trimethyl silyl propionic acid sodium salt, w/v) was added. The mixture was vortexed at room temperature for 1 min, ultrasonicated for 20 min, and centrifuged at 13,000 rpm at room temperature for 5 min. Eight hundred µl of the supernatant was transferred to a 5 mm NMR tube. NMR measurements were done as mentioned in our previous studies.^{27, 28}

2.4 Data analysis

The ¹H NMR spectra were automatically reduced to ASCII (v. 3.7, Bruker Biospin). Spectral intensities were scaled to trimethylsilyl propionid acid sodium salt (TMSP) and reduced to integrated regions of

equal width (0.04) corresponding to the region of δ 0.4 – δ 10.0. The data were normalised to total intensity. The region of δ 4.7 – δ 4.9 was excluded from the analysis because of the possible residual signal of water. Principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were performed with the SIMCA-P software (v. 11.0, Umetrics, Umeå, Sweden) by using unit variance (UV) scaling method.

3 Results and Discussion

The ^1H NMR spectra of methanol water extracts for the healthy aerial parts (leaves and petioles) and roots of red radish (*Raphanus sativus*) were studied. Based on NMR spectroscopy of roots and leaves, a number of metabolites were identified including amino acids, organic acids, carbohydrates, flavanoids, phenylpropanoids and glucosinolates. The signals of alanine, threonine, valine, fumaric acid, adenine, gallic acid, malic acid, glutamate, glutamine, acetate and GABA (γ -aminobutyric acid), were identified in the spectral region of organic and amino acids. Five phenylpropanoids (feruloyl malate, caffeoyl malate, sinapoyl malate, 5-hydroxyferuloyl malate, coumaroyl malate) and two glucosinolates (glucobrassicin and neoglucobrassicin) were identified in aromatic region as previously reported by our group.^{24, 28, 354} In addition, flavonoids, quercetin-7-glucoside (quercimeritrin) at δ 6.47 (d, $J = 2.2$), δ 6.75 (d, $J = 2.2$), δ 7.07 (dd, $J = 8.0, 2.1$), δ 6.81 (d, $J = 8.3$), δ 7.21 (d, $J = 2.2$), and two kaempferol analogues at δ 8.05 (d, $J = 9.2$), δ 6.99 (d, $J = 9.2$), δ 6.80 (d, $J = 2.1$), δ 6.58 (d, $J = 2.2$) and δ 8.06 (d, $J = 9.2$), δ 7.03 (d, $J = 9.2$), δ 7.26 (d, $J = 2.2$), δ 7.15 (d, $J = 2.2$) were identified. These assignments were confirmed by diverse 2D NMR spectra including J-resolved, COSY, HSQC, HMBC, and by the comparison with reference compounds.

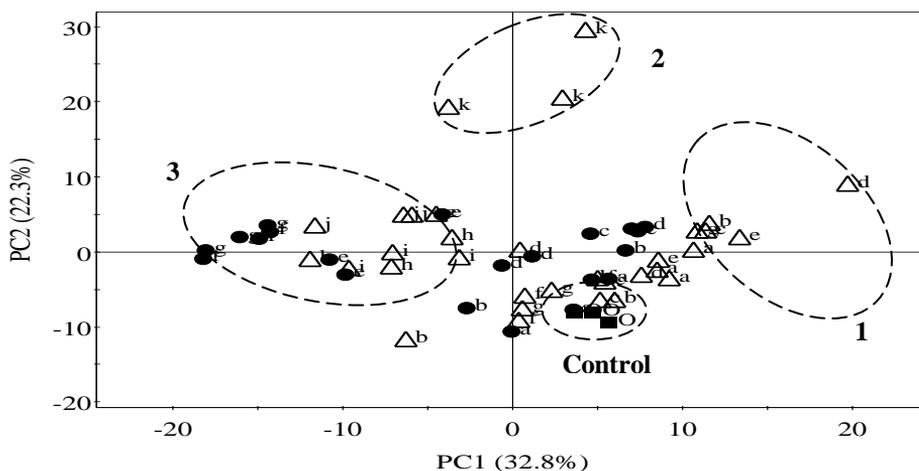
Principal component analysis (PCA) enables straightforward visualization of data similarities or differences in the data set.³⁵ Our study results were assessed by PCA and also by direct visual comparison of ^1H NMR spectra of different treatments. Using the data collected during this study, we examined the effect of storage time and temperature on the radish metabolome. Amino acids, organic acids, sugars, phenylpropanoids, flavonoids and glucosinolates were found to be the major discriminating metabolites (**Table 1**).

The leaves and roots spectra are compared in PCA. From these PCA results it is also concluded that the change in metabolites of leaves

is much higher as compared with the change in metabolites of roots, due to the diversity of compounds in leaves. This shows a clear separation of leaves and roots in two different groups. Carbohydrates are present in high amounts in roots as compared with leaves; on the other hand leaves show a high concentration of other primary and secondary metabolites.²⁷ So for the comparison of metabolite profiles, ¹H NMR spectra of radish roots and leaves were analyzed separately by PCA. A clear difference in both cases, among different treatments was observed, but still due to a large number of samples, there was a massive overlapping of different treatments in PCA score plot. So to extract the common factors, the samples stored at room temperature and at 4 °C along with control, were separately investigated (**Figure 1 A, B**). While the samples stored at -20 °C and -80 °C were assessed together, along with control in PCA (**Figure 2 A, B**).

After three days of storage at room temperature, yellowing of radish leaves was observed, showing the loss of pigments, similar results were reported for rocket salad (*Eruca sativa* Mill.).¹²⁸ At 4 °C yellowing of leaves was less discernible until 28 days of storage, while at chilling temperatures (-20 °C and -80 °C) it was negligible as compared with samples stored at room temperature. Plants integrate roots in resistance and tolerance mechanisms of leaf defence. Roots are not only a storage site for plant metabolites but also provide a backup supply of primary metabolites to the plant for secondary metabolite production⁴³⁰. Overall the roots of radish plants stored at different temperatures showed similar changes during storage as that to plant leaves, except those stored at 4 °C. At 4 °C an increase in phenylpropanoids, and a decrease in fumaric acid and adenine is observed with increasing storage time (**Figure 1 A, B**).

The metabolomic changes still continue even after harvesting the plants, e.g. broccoli undergoes losses of sugars, organic acids, and proteins within the first 6 hours of harvest, which is followed by increase in the free sugars, amino acids, and organic acids.^{41, 254} A continuous change in radish metabolites is observed during storage at 4 °C and room temperature storage, as the result of postharvest physiological stress due to non-availability of nutrients. Leaves show high amount of phenylpropanoids, gallic acid and malic acid at early storage, while amino acids, organic acids and glucosinolates are found to be discriminating metabolites for late storage at room temperature and 4 °C. In roots, phenolic compounds are found in high amounts at late storage, but interestingly in both types of samples glucosinolates are found to increase in late storage (**Figure 1**).



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Figure 1 (A). Score plot (PC1 vs PC2) of PCA for radish leaves, based on whole range of ^1H NMR signals (δ 0.0 – δ 10.0); control (■), samples stored at 4 °C (Δ) and room temperature (●); control (O); 2 h (a); 4 h (b); 6 h (c); 12 h (d); 24 h (e); 2 d (f); 3 d (g); 7 d (h); 14 d (i); 21 d (j); 28 d (k); **1** = feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate, coumaroyl malate, and caffeoyl malate, kaempferol, quercetin, gallic and malic acid; **2** = Fumaric acid, glucose, sucrose, adenine; **3** = threonine, alanine, valine, glutamine, glutamate, glucobrassicin, and neoglucobrassicin.

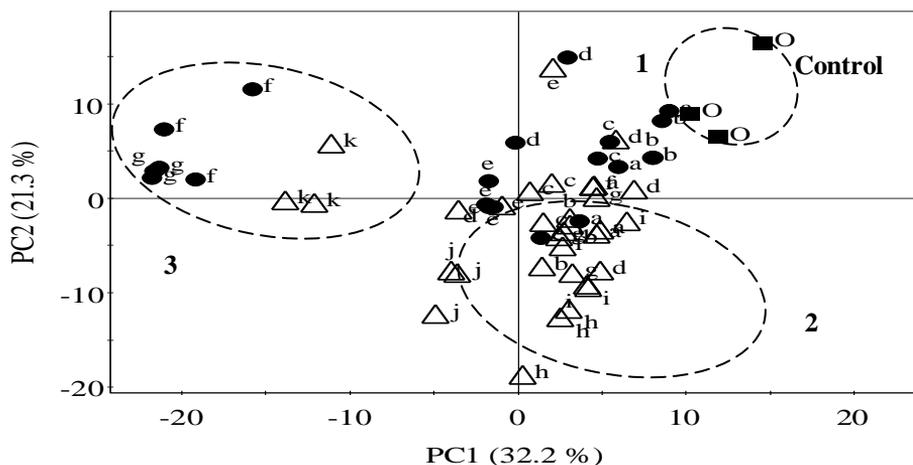


Figure 1 (B). Score plot (PC1 vs PC2) of PCA for radish roots, based on whole range of ^1H NMR signals (δ 0.0 – δ 10.0); control (■), samples stored at 4 °C (Δ) and room temperature (●); control (O); 2 h (a); 4 h (b); 6 h (c); 12 h (d); 24 h (e); 2 d (f); 3 d (g); 7 d (h); 14 d (i); 21 d (j); 28 d (k); **1** = Fumaric acid, adenine; **2** = glucose, malic acid, sinapoyl malate; **3** = feruloyl malate, 5-hydroxyferuloyl malate, coumaroyl malate, and caffeoyl malate, kaempferol, quercetin, gallic acid, sucrose, threonine, alanine, valine, glutamine, glutamate, glucobrassicin, and neoglucobrassicin.

In case of cold stored samples ($-20\text{ }^{\circ}\text{C}$ and $-80\text{ }^{\circ}\text{C}$) discrimination in metabolites is observed between early storage and final day storage due to the presence of high amounts of sucrose, phenolics and glucosinolates in early storage samples, and high amounts of amino acids, organic acids at late storage. An increase in sucrose and amino acids is known as response to cold stress.⁴¹⁸ A decrease of glucose is also observed during storage.

From the PCA of the results of this study, it can be concluded that the decrease in phenolic content differs for different storage temperatures. In the PCA score plot, all the samples of the first 24 hours were found near to control in PC1 and PC2. If focusing only at the phenolic region the PCA shows that a high amount of phenylpropanoids and flavonoids is characteristic for the control and early storage (e.g. **Figure 2**).

When only the initial storage hours are analysed, it is clear that by increasing time an increase in phenolics and glucosinolates occurs during the early storage. The increase of phenolics and glucosinolates in cold stored samples may be correlated to the physiological stress due to the chilling injury and non-availability of the nutrients in the start of cold storage.^{11, 128} The phenylpropanoids, especially ferulic acid accumulation are thought to result in cell wall rigidity to protect it from chilling injury.⁴³¹ After some time production of phenolics and glucosinolates stalls,¹²⁸ though still an increase of amino acids, and glucose is observed (**Figure 3**).

A similar behaviour was observed for leaves and roots. Cold storage may improve phenolics related quality¹¹ as in some cases low temperature increases anthocyanins, and hydroxycinnamic acid derivatives.¹¹ Accumulation of phenylpropanoids in *Arabidopsis* at low temperature is also reported.⁴¹⁸ An increase in phenylpropanoids during early chilling ($-20\text{ }^{\circ}\text{C}$ and $-80\text{ }^{\circ}\text{C}$) storage may protect cells against frost-induced oxidative stress, scavenging hydrogen peroxide diffusing across membranes.⁴³¹

At later storage the plant tissue gets frozen and biological activity may stop but chemical activity continues and leads to further metabolomic changes. Although a separation for different treatments is observed in PCA, still some clear markers for the different treatments and for the time course would be important. Therefore a supervised multivariate analysis method PLS-DA was applied to the data. The grouping was made on the basis of different time periods (twelve groups) (**Figure 4, 5**) and temperatures (five groups) (**Figure 6, 7**).

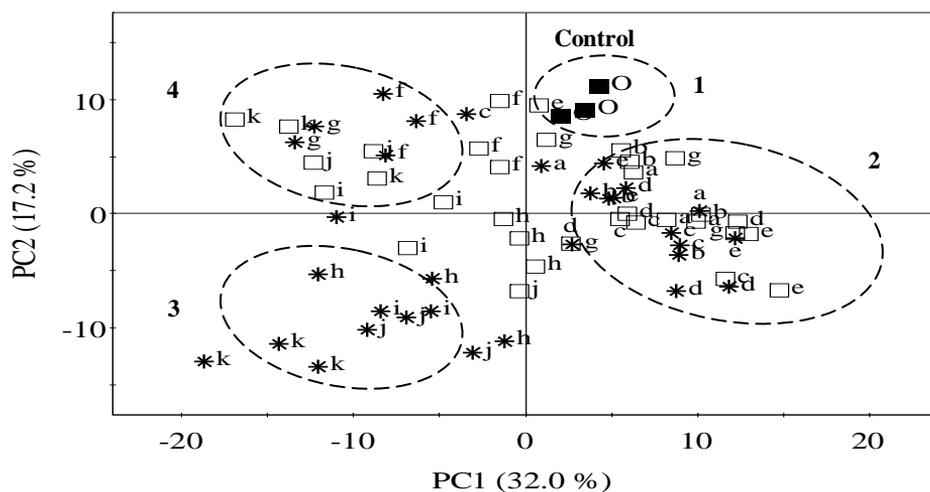


Figure 2 (A). Score plot (PC1 vs PC2) of PCA for radish leaves, based on whole range of ^1H NMR signals (δ 0.0 – δ 10.0); control (■), samples stored at -20°C (*) and -80°C (□); control (O); 2 h (a); 4 h (b); 6 h (c); 12 h (d); 24 h (e); 2 d (f); 3 d (g); 7 d (h); 14 d (i); 21 d (j); 28 d (k); **1** = glucose; **2** = feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate, coumaroyl malate, and caffeoyl malate, kaempferol, quercetin, sucrose, adenine, glucobrassicin and neoglucobrassicin; **3** = alanine and threonine, **4** = valine, glutamine, glutamate, and malic acid.

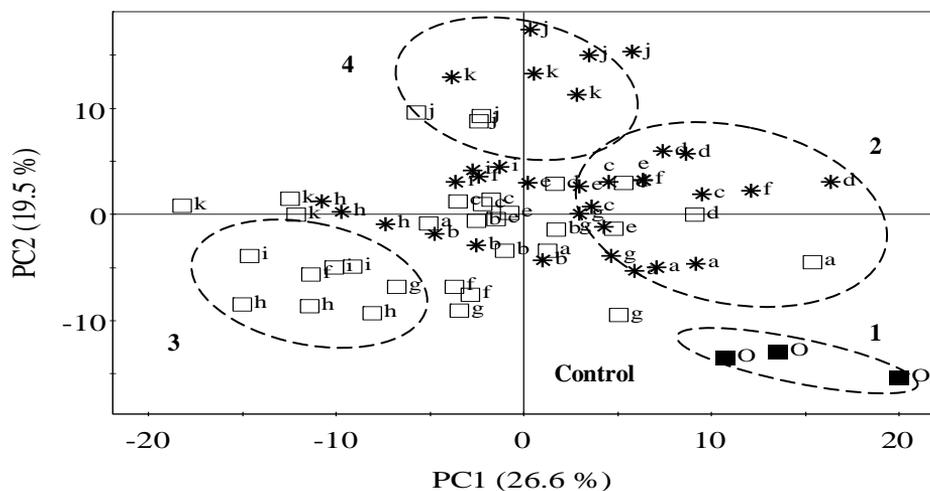


Figure 2 (B). Score plot (PC1 vs PC2) of PCA for radish roots, based on whole range of ^1H NMR signals (δ 0.0 – δ 10.0); control (■), samples stored at -20°C (*) and -80°C (□); control (O); 2 h (a); 4 h (b); 6 h (c); 12 h (d); 24 h (e); 2 d (f); 3 d (g); 7 d (h); 14 d (i); 21 d (j); 28 d (k); **1** = glucose; **2** = feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate, coumaroyl malate, and caffeoyl malate, adenine, kaempferol, quercetin, glucobrassicin and neoglucobrassicin; **3** = sucrose; **4** = alanine, threonine, valine, glutamine, glutamate, and malic acid.

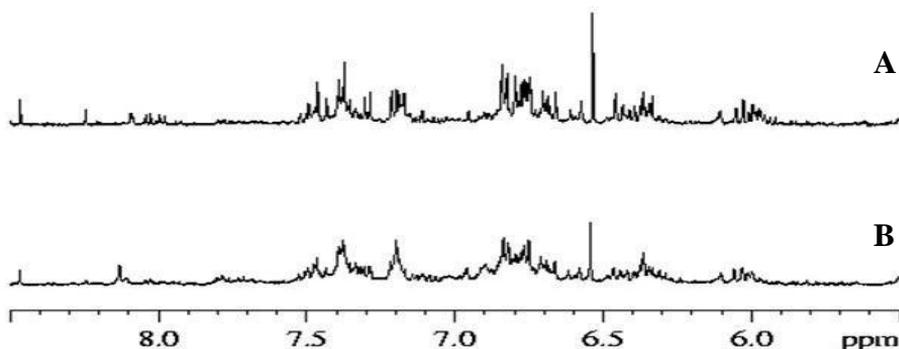


Figure 2. ^1H NMR spectra of aerial parts of red radish (petioles and leaves) stored at 4 °C for 12 hours (A) and 28 days (B).

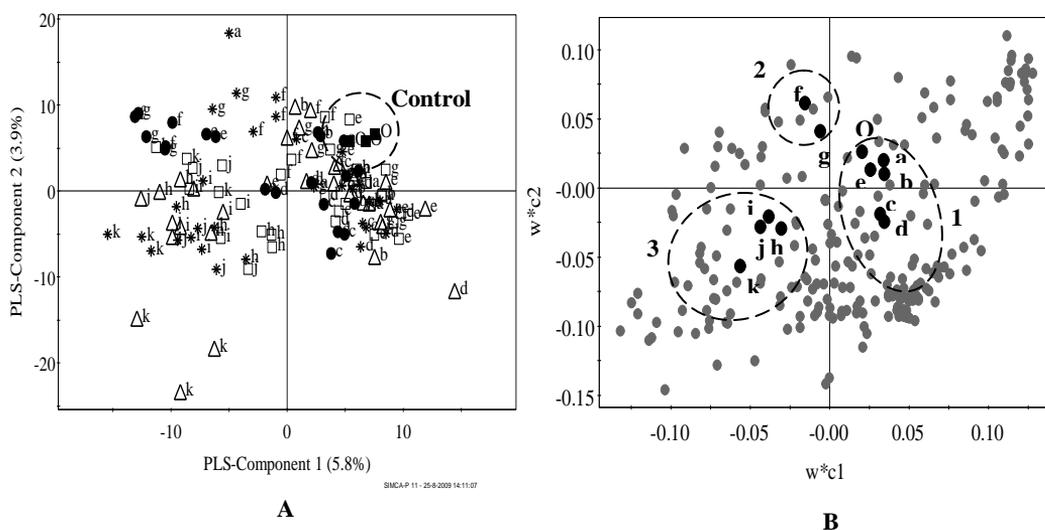


Figure 4. Score plot (PLS-Component 1 vs PLS-Component 2) (A) and loading (B) of PLS-DA, for effect of storage time on radish leaves, based on whole range of ^1H NMR signals (δ 0.0 – δ 10.0); control (■), samples stored at room temperature (●); at 4 °C (Δ); at -20 °C (*) and -80 °C (\square); control (O); 2 h (a); 4 h (b); 6 h (c); 12 h (d); 24 h (e); 2 d (f); 3 d (g); 7 d (h); 14 d (i); 21 d (j); 28 d (k); **1** = valine, feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate, coumaroyl malate, and caffeoyl malate, kaempferol, quercetin, and neoglucobrassicin, adenine, glucose, glucobrassicin; **2** = alanine, glutamine, glutamate; **3** = threonine, sucrose, and malic acid.

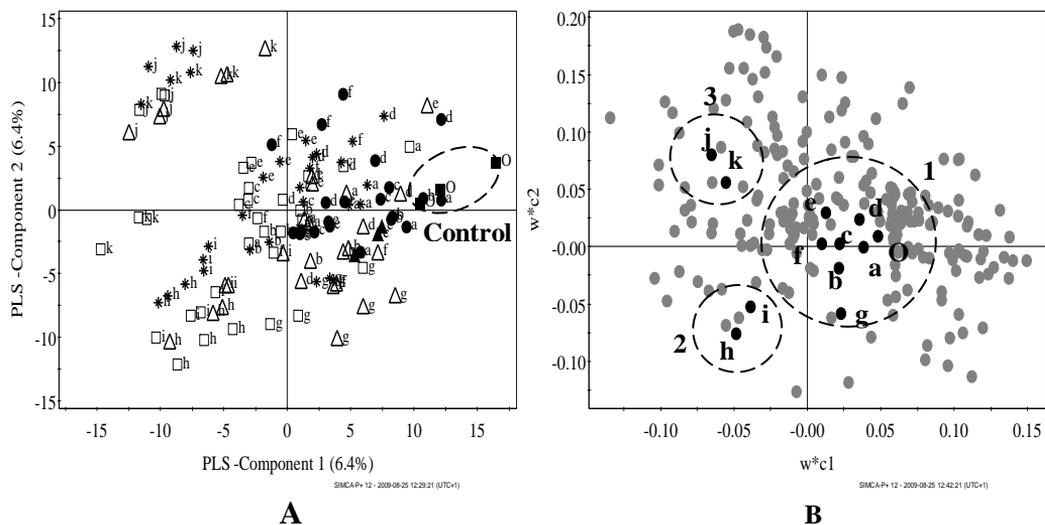


Figure 5. Score plot (PLS-Component 1 vs PLS-Component 2) (A) and loading (B) of PLS-DA, for effect of storage time on radish roots, based on whole range of ^1H NMR signals (δ 0.0 – δ 10.0); control (■), samples stored at room temperature (●); at 4 °C (Δ); at –20 °C (*) and –80 °C (□); control (O); 2 h (a); 4 h (b); 6 h (c); 12 h (d); 24 h (e); 2 d (f); 3 d (g); 7 d (h); 14 d (i); 21 d (j); 28 d (k); **1** = adenine, glucose, fumaric acid, feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate, and caffeoyl malate, kaempferol, quercetin, glucobrassicin and neoglucobrassicin; **2** = alanine, glutamine, glutamate, valine, and sucrose; **3** = threonine, malic

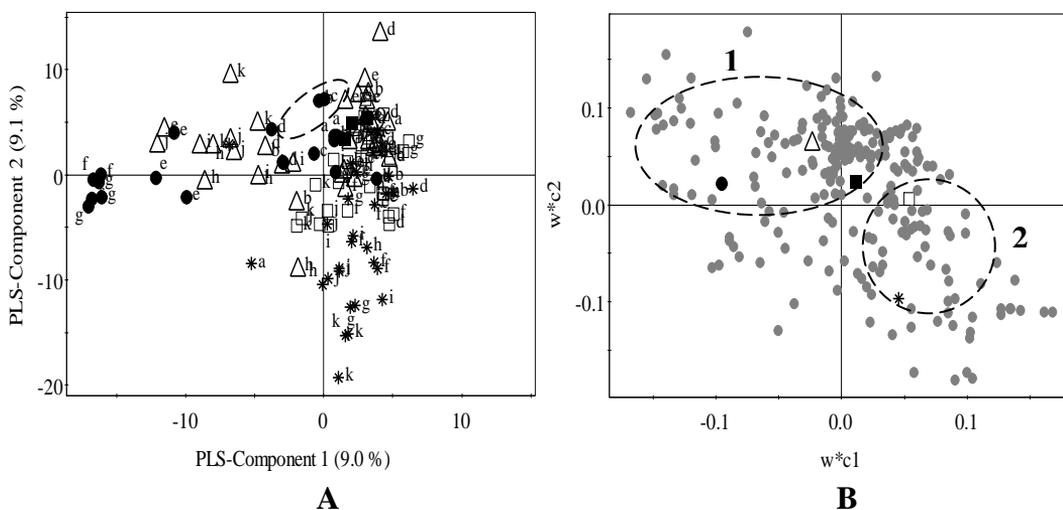


Figure 6. Score plot (PLS-Component 1 vs PLS-Component 2) (A) and loading (B) of PLS-DA, for effect of storage temperature on radish leaves, based on whole range of ^1H NMR signals (δ 0.0 – δ 10.0); control (■), samples stored at room temperature (●); at 4 °C (Δ); at –20 °C (*) and –80 °C (□); control (O); 2 h (a); 4 h (b); 6 h (c); 12 h (d); 24 h (e); 2 d (f); 3 d (g); 7 d (h); 14 d (i); 21 d (j); 28 d (k); **1** = alanine, valine, threonine, glutamine, glutamate, glucobrassicin, sucrose, α -glucose, feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate, coumaroyl malate, and caffeoyl malate, kaempferol, quercetin, and neoglucobrassicin; **2** = adenine, β -glucose, and malic acid.

The results of PLS-DA for leaves (**Figure 4 A, B**) and roots (**Figure 5 A, B**) supports the conclusions of the PCA and further explain that with increasing time first an increase in secondary metabolites is observed, followed by a decrease in these compounds. This decrease may be correlated with the release of breakdown products of plant metabolites.¹²⁸ High amounts of primary metabolites including sugars, amino acids and organic acids are observed in late storage samples (**Figure 4, 5**).

The samples stored at 4 °C show less separation in PLS-DA from control as compared with that stored at room temperature, while samples stored at -80 °C shows less separation in PLS-DA from control as compared with that at -20 °C. Comparison of leaves stored at different temperatures (**Figure 6**) shows discrimination for samples stored at room temperature, due to glutamine, glutamate, threonine, alanine and glucobrassicin, while -20 °C and -80 °C samples were separated by the samples at room and 4 °C by adenine, malic acid, and β -glucose signals. A high amount of phenylpropanoids, flavonoids, neoglucobrassicin, malic acid, and sucrose is characteristic for the leaf samples stored at 4 °C (**Figure 6**).

Low temperature acclimation induces sucrose and organic acids in plant. This increases the plant cell tolerance against dehydration and freezing injury.^{432, 433} The increase of organic acids and amino acids in later storage shows the selectivity and shift of plant metabolomic pathways, most suited for its survival. Loss of glucose and secondary metabolites in the late storage may also results in the increase in amino acids under storage.⁴¹⁷

Storage at -80 °C exhibits less metabolomic changes (**Figure 3 A, B**) by decreasing tissue respiration at cold temperature.⁹⁶ The root samples stored at room temperature, 4 °C and control show separation in PLS-DA (**Figure 7**) due to glucosinolates, phenylpropanoids, sucrose, valine, glutamine, glutamate, and fumaric acid, while the samples stored at -20 °C and -80 °C show discrimination due to alanine, threonine and adenine. Different preharvest and postharvest factors, including storage temperature, significantly affect phenolic degradation or stability.¹¹ A decrease in anthocyanins in red radish was observed during storage, both in light and dark conditions,⁴³⁴ while decreasing the storage temperatures the loss of phenolic is reduced.¹¹ With the results of NMR based metabolomic characterization and visual appearance of radish leaves and roots, the samples stored at low temperature were found closest to

control. By visual assessment the quality of radish stored at 4 °C were found of acceptable quality, even after 28 days of storage, but samples stored at room temperature were not acceptable any more after three days of storage. Samples stored at -20 °C and -80 °C looked fresh but after defrosting due to thawing effect, radish roots and leaves were not acceptable for consumption.

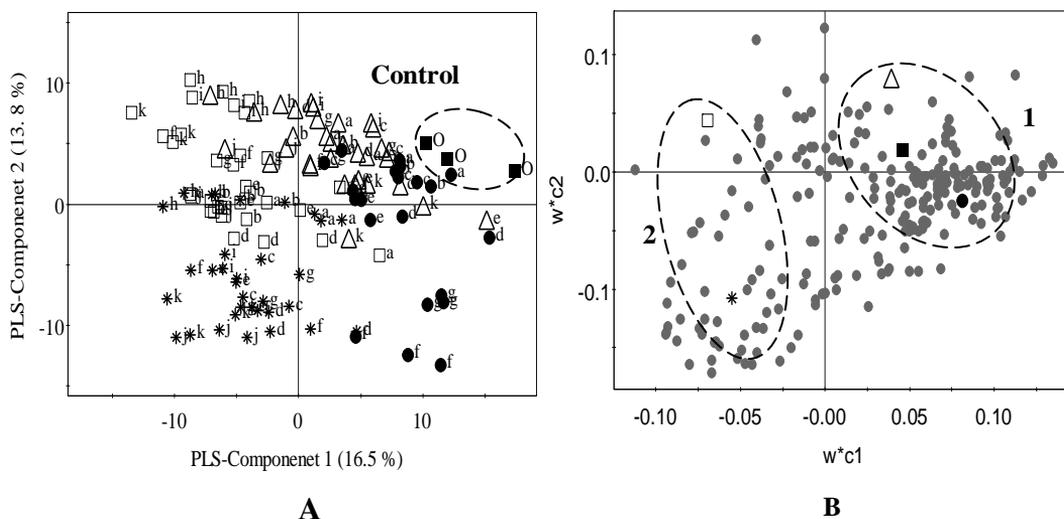


Figure 7. Score plot (PLS-Component 1 vs PLS-Component 2) (A) and loading (B) of PLS-DA, for effect of storage temperature on radish roots, based on whole range of ^1H NMR signals (δ 0.0 – δ 10.0); control (■); room temperature (●); 4 °C (Δ); -20 °C (*); -80 °C (□); control (O); 2 h (a); 4 h (b); 6 h (c); 12 h (d); 24 h (e); 2 d (f); 3 d (g); 7 d (h); 14 d (i); 21 d (j); 28 d (k); **1** = glucose, and malic acid, sucrose, valine, glutamine, glutamate, fumaric acid, feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate, coumaroyl malate, and caffeoyl malate, kaempferol, quercetin, glucobrassicin, neoglucobrassicin; **2** = alanine, threonine, adenine.

4 Conclusion

Multivariate data analysis method is a promising method to find biomarker compounds relative to storage at different time and storage. Radish roots show least metabolomic changes as compared with leaves, when compared in PCA. While focusing on effect of temperature, least metabolomic changes were observed in the samples stored at $-20\text{ }^{\circ}\text{C}$ and $-80\text{ }^{\circ}\text{C}$, these temperature conditions are found comparatively best for experimental purpose and it is advantageous to keep the vegetable samples at $-80\text{ }^{\circ}\text{C}$, for research sampling. But in this case the post-storage physical quality of radish is not acceptable as freezing destroys the structure and after thawing massive changes in the metabolome occur. Adenine, malic acid in leaves while adenine, alanine and threonine in roots, are found as discriminating metabolites for the storage temperature of $-20\text{ }^{\circ}\text{C}$ and $-80\text{ }^{\circ}\text{C}$. A rapid increase of glucosinolates until the later storage time is studied at room temperature and $4\text{ }^{\circ}\text{C}$. While at $-20\text{ }^{\circ}\text{C}$ and $-80\text{ }^{\circ}\text{C}$ an increase in these compounds is observed at early storage only. *Brassica* vegetables offer a good basis for the development of healthy food, however vegetable storage has a critical role in the preservation of these compounds so further research is needed to study these compounds in relation to their breakdown products.

Table 1 – A: Effect of different temperatures on the metabolites of red radish aerial parts (petioles and leaves) at 20 °C, 4 °C, –20 °C and –80 °C. Increase until late storage (+); decrease until late storage (–); increase in initial storage and decrease in late storage (+–).

	20 °C	4 °C	–20 °C	–80 °C
Adenine	+	+	+	+ –
Fumaric acid	+	+	+ –	+ –
Galic acid	–	+ –	–	–
Glutamine and glutamate	+	+	+ –	+ –
Malic acid	–	–	+ –	+ –
Alanine and threonine	+	+	+	+ –
Valine	+	+	+ –	+ –
Glucose	+	+	–	–
Sucrose	+	+	+ –	+ –
Glucobrassicin and neoglucobrassicin	+	+	+ –	+ –
5-hydroxyferuloyl -, caffeoyl -, coumaroyl -, feruloyl -, and sinapoyl malate	+ –	+ –	+ –	+ –
Flavonoids	+ –	+ –	+ –	+ –

Table 1 – B: Effect of different temperatures on the metabolites radish roots at 20 °C, 4 °C, –20 °C and –80 °C. Increase until late storage (+); decrease until late storage (–); increase in initial storage and decrease in late storage (+ –).

	20 °C	4 °C	–20 °C	–80 °C
Adenine	–	–	–	–
Fumaric acid	–	–	–	–
Galic acid	+	+	+	–
Glutamine and glutamate	+	+	+	+ –
Malic acid	+	+	+	+ –
Alanine and threonine	+	+	+	+ –
Valine	+	+	+	+ –
Glucose	+ –	+ –	–	–
Sucrose	+	+	+ –	+
Glucobrassicin and neoglucobrassicin	+	+	+ –	+ –
Sinapoyl malate	+ –	+ –	+ –	+ –
5-Hydroxyferuloyl -, caffeoyl -, coumaroyl -, and feruloyl malate	+	+	+ –	+ –
Flavonoids	+	+	+ –	+ –