Metabolomic Approach: Postharvest Storage Stability of Red Radish (*Raphanus sativus* L.)

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Summary: Post harvest storage of vegetables at different temperature for consumption is commonly practiced that need standardization. Among vegetables, red radish (*Raphanus sativus* L.) is a well known and commonly consumed vegetable all over the world. Its bioactive or nutritional constituents include a wide range of metabolites including, glucosinolates, phenolics, amino acids, organic acids, and sugars. However, many of these metabolites are not stable and can easily be degraded or modified during storage. In order to investigate the metabolomic changes during post harvest storage, radish samples (intact roots and aerial parts) were subjected to four different storage temperatures above and below 0 °C (20 °C, 4 °C, –20 °C, and –80 °C), for a maximum of 28 days. ^{1}H-NMR and two-dimensional NMR spectra data resulting from the analysis of the different samples were subjected to principal component analysis (PCA) to investigate any possible metabolomic changes.

A profound chemical alteration was observed in primary and secondary metabolites. Glucosinolates, phenylpropanoids, organic acids, amino acids, and sugars were found to be the discriminating metabolites for the storage effect. Initially, an increase in secondary metabolites (phenolics and glucosinolates) was observed, but levels of these compounds decreased in later stages, probably due to the breakdown of these products. Whereas late storage samples contained high amounts of amino acids [alanine, valine, threonine, (γ-amino-butyric acid / GABA)] and some glucosinolates (glucobrassicin, neoglucobrassicin). This phenomenon was pronounced at room temperature as compared to other storage temperatures. Interestingly even at lower and freezing temperatures metabolomic changes in these biological samples were observed. The least metabolomic changes were observed at samples stored at –80 °C. While studying temperature dependent metabolomic changes, high levels of glucose, adenine, alanine, threonine and GABA were observed during storage below 0 °C, especially noticeable at –20 °C.

Keywords: *Raphanus sativus*; Metabolomics; Storage effect; Postharvest storage, Multivariate data analysis.

Introduction

The evidence for the importance of health-promoting bioactive compounds present in Brassicaceae vegetables has increased in the last few years [1]. Because of the nutritional importance of Brassicaceae metabolites, there has been an increasing interest in the evaluation of the behavior of these compounds after postharvest treatments [2]. Radish is considered as to be the most important and widely known vegetables of the Brassicaceae [3, 4], and is considered to have health benefits due to the presence of sugars, amino acids, organic acids, phenolics, and glucosinolates [5-8]. Additionally radish is considered to be a well-established model system for plant research [9, 10]. Like other vegetables, radish can be preserved by storage, pickling, canning or drying [11]. However their chemical compositions, especially the phenolics are easily affected by pre-harvest agronomic and post-harvest processing and storage conditions [12, 13].

The diverse post-harvest processing and storage methods have been reported to retard the degradation of bioactive compounds, while retaining the other quality attributes of different vegetables [14-16]. The post-harvest storage method chosen for vegetables or research samples (e.g. low temperature storage, freezing, room temperature), industrial processing (drying, blanching, canning etc.), and different cooking procedures play an important role in the level and type of degradation suffered by Brassicaceae bioactive components [17].

It is possible to prevent the loss of nutritional value by using lower temperatures [12, 18], as it results in a decreased metabolic rate, thus preserving radish quality [19]. While on the other hand storage at chilling temperature can have positive and negative effects on vegetables, depending on the commodity and the storage temperature [13]. The activation of various stress-inducible pathways [20]

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and diverse kinetics in the accumulation of numerous metabolites are an advantageous for plants to get prepared and face low temperature stress conditions [21].

Effect of post harvest storage conditions, especially cold storage, on the plant metabolome has always been a controversial issue. These changes in vegetables or research samples may occur due to pure chemical conversion (fastest at higher temperature) or to residual metabolism of the plants and plant cells after harvesting. The latter includes the plant response to wounding, infection, draught and temperature, which is quite complex and less predictable as one or more responses may be active depending on the conditions.

The comprehensive quantitative and qualitative metabolomic analysis of cells, tissues and/or organisms is an ambitious goal [18]. However, the knowledge of metabolomic components, which are viewed as the end products of genetic expression, can be used to define the biochemical environment of cells or tissues. Recently the metabolomic techniques, providing broad biochemical spectrum of an organism [22], has been successfully applied to study the phytochemical changes in plants [23]. As the diversity in plant metabolites is too large and so with the range of volatility, polarity, solubility, chromatographic behavior of different groups, the detectability of these compounds differs and so currently no single analytical tool can provide complete metabolomic information of plants, [24]. Therefore, the selection of the most suitable analytical method is generally a compromise between speed, selectivity and sensitivity [23].

Nuclear magnetic spectroscopy (NMR) based metabolomics is becoming increasingly recognized in research and development as a highly reproducible and quantitative method. Although ^{1}H-NMR is not as sensitive as some other analytical methods, such as HPLC, MS, etc. but being a non destructive and comprehensive analytical tool, large number and diverse groups of compounds can be detected by NMR, in a single run [18]. So NMR seems an optimum choice to study the overall major changes in the metabolome during cold storage.

A lot of research work has been done on the storage stability of vegetables after pre-treatment (e.g. blanching, packing, UV etc.) but there is a need to study the effect of different storage temperatures, especially freezing temperatures (e.g. – 20 ^{o}C and – 80 ^{o}C).

In present case study of radish, the situation is quite special as the roots and green parts of the plant remain intact, since this is what consumed as a vegetable or can be stored for research for further use of quality evaluation or metabolomic characterization, etc. The objective of the present research was to investigate the phytochemical changes in radish metabolome at different time points and temperatures, either stored for food usage or research purposes, focusing particularly on compounds related to the quality of the plant as a foodstuff. To analyze the metabolomic alterations, ^{1}H-NMR and 2D–NMR spectra were used in combination to principal component analysis (PCA).

Results and discussion

The methanol water extracts of healthy aerial parts (leaves and petioles) and roots of red radish (*Raphanus sativus*) were studied by ^{1}H-NMR. Based on ^{1}H-NMR spectra, a range of metabolites were identified, that includes carbohydrates, organic acids, amino acids, phenylpropanoids, flavanoids and glucosinolates. The signals of alanine, threonine, valine, acetate, fumaric acid, glutamate, glutamine, adenine, gallic acid, malic acid and GABA, were detected in the spectral region of organic and amino acids. Five phenylpropanoids (sinapoyl malate, feruloyl malate, caffeoyl malate, coumaroyl malate, 5-hydroxyferuloyl malate) and two glucosinolates (glucobrassicin and neoglucobrassicin) were identified in aromatic region as previously reported by our group [25, 26]. In addition, flavonoids, quercetin-7-glucoside (quercimeritrin) at 6.47 (d, J = 2.2), 6.75 (d, J = 2.2), 7.07 (dd, J = 8.0, 2.1), 6.81 (d, J = 8.3), 7.21 (d, J = 2.2), and two kaempferol analogues at 8.05 (d, J = 9.2), 6.99 (d, J = 9.2), 6.80 (d, J = 2.1), 6.58 (d, J = 2.2) and 8.06 (d, J = 9.2), 7.03 (d, J = 9.2), 7.26 (d, J = 2.2), 7.15 (d, J = 2.2) were identified. These assignments were confirmed by two dimensional NMR spectra (i.e. J-resolved, COSY, HSQC and HMBC).

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, flavonoids, phenylpropanoids and glucosinolates were found to be the major discriminating metabolites, acting differently according to their storage times and temperatures (Table-1 and 2). Although these differences can be observed by visual comparison of their ^{1}H-NMR spectra (Fig. 1), but it is difficult to draw conclusions due to the large number of samples and a number of signals in each spectrum. It is thus necessary to use Principal component analysis (PCA), which enables the straightforward visualization of similarities or variations in the data set [18] obtained in this study.

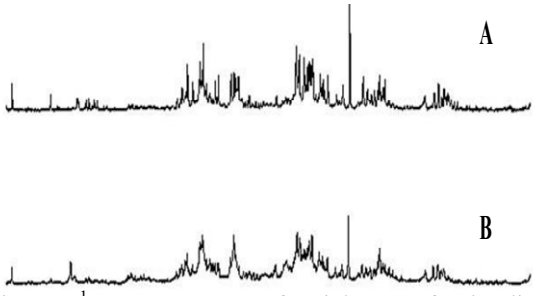

Fig. 1: ^{1}H-NMR spectra of aerial parts of red radish (petioles and leaves) stored at 4 ^{o}C for 12 h (A) and 28 days (B).

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

	20 ^{o}C	4 ^{o}C	–20 ^{o}C	–80 ^{o}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-2: Effect of different temperatures on the metabolites in radish roots at 20 ^{o}C, 4 ^{o}C, –20 ^{o}C and –80 ^{o}C. Increase until late storage (+); decrease until late storage (–); increase in initial storage and decrease in late storage (+ –).

	20 ^{o}C	4 ^{o}C	–20 ^{o}C	–80 ^{o}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	+	+	+–	+–

After three days of storage at room temperature, yellowing of radish leaves was observed, showing the loss of pigments. At 4 ^{o}C yellowing of leaves was less visible until 28 days of storage, while at chilling temperatures (–20 ^{o}C and –80 ^{o}C) no effect was observed within the 28 day period established for the study.

To analyze the effect of temperature and storage time on the metabolite profile, spectra of leaves and roots were compared using PCA. From PCA results it was concluded that the change in metabolites of leaves is much higher than the change in metabolites of roots. A possible explanation for this is might be that the leaves has much higher diversity of compounds as compared to roots, while the roots are mainly a storage site for plant metabolites that also gives a backup supply of primary metabolites needed for secondary metabolite production (mostly in leaves) [27]. Furthermore, 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 [24]. This results a clear separation of leaves and roots in two different groups. Thus, for the comparison of metabolite profiles, ^{1}H-NMR spectra of radish roots and leaves were analyzed separately by PCA. A clear difference in all cases among different treatments was observed. The separation was especially clear in the case of samples stored above and below 0 ^{o}C, when assessed by drawing the score plot between PC1 and PC3 for leaves and PC1 and PC2 for roots. However due to the large number of samples, there was a massive overlapping of different treatments in the PCA score plot. So in order to extract the factors affecting this separation, the score value for each variable from score scatter plot of PCA was used to draw multi score plots (Fig. 2 A, B, C). A similar method was used to evaluate the effect of time and temperature on radish roots, by comparing PC1 and PC2 (Fig. 3 A, B, C).

The results thus obtained showed that metabolomic changes in plants can continue after harvesting depending on the time and temperature of their storage. This has been observed in broccoli, for example, which was reported to show a decrease in levels of sugars, organic acids, and proteins within the first 6 h of harvest, followed by an increase in the free sugars, amino acids, and organic acids [28, 29]. In this study, the PCA revealed the phenolic content to be a discriminating factor for different storage times and temperatures. On this basis, storage time can be categorized as initial, middle and final storage time (Fig. 2 A). A high amount of phenolics was observed at initial storage time while the levels of glucosinolates and amino acids showed an increasing trend throughout time, which could be interpreted to be a result of the stress response to blockage of nutrient supply and presence of draught conditions.

A clear discrimination between samples stored below 0 ^{o}C (– 20 ^{o}C and – 80 ^{o}C) and above 0 ^{o}C (4 ^{o}C and 20 ^{o}C) was observed. In this case a continuous change in radish metabolites was observed between early storage and the final day of storage, with high amounts of glucose, sucrose and phenolics in early storage samples. Whereas late storage samples contained high amounts of amino acids (alanine, valine, threonine, GABA) and glucosinolates (glucobrassicin, neoglucobrassicin). For aerial parts of radish, the highest metabolomic changes were observed at room temperature (20 ^{o}C) within three days of storage, as compared to other temperatures even after 28 days of storage time (Fig. 2 B). The least metabolomic changes were observed at samples stored at – 80 ^{o}C. While studying temperature dependent metabolomic changes, high levels of glucose, adenine, alanine, threonine and GABA were observed during storage below 0 ^{o}C, especially noticeable at – 20 ^{o}C. This is surprising but this increase of amino acids is might be associated with a response to cold stress, as reported previously [21].

Leaves exhibited high amount of phenylpropanoids and malic acid at early storage, while amino acids, organic acids and glucosinolates were found to be discriminating metabolites for late storage at different temperatures (Fig. 2) as a result of postharvest physiological stress due to the non-availability of nutrients. If focusing only on the phenolic region, PCA shows a high amount of phenylpropanoids and flavonoids for the control and early storage samples. Circumscribing the analysis to the initial storage h, it is clear that phenolics and glucosinolates increased with time during the early storage. The increase of phenolics and glucosinolates in cold stored samples may be correlated to the physiological stress caused by chilling injury and non-availability of the nutrients in the initiation of cold storage [13, 30]. A possible explanation of this can be found in the fact that hydroxycinnamic acids, especially ferulic acid accumulation are thought to provide cell wall rigidity, which could protect the plant from chilling injury [31]. In this process, after some time, production of phenolics and glucosinolates stalls [30] though an increase of amino acids and glucose can still be observed (Fig. 2, 3). Later on the plant tissue freezes and biological activity may stop completely even though chemical changes can still continue leading to further metabolomic changes.

In the case of roots, an increase in storage time produced an increase in phenolics, glucosinolates and amino acids at all temperatures except at – 80 ºC., whereas on this temperature, least discrimination between samples was observed as compared to other storage temperatures (Fig. 3). The increase in aforementioned compounds was observed until the first three days of storage after which these compounds decreased while the concentration of glucose increased. However, after this, the level of phenolics, glucosinolates and amino acids was increased again throughout the 28-day storage period, in the samples stored at 4 ^{o}C and – 20 ^{o}C. In this case glucose is decreased in PC1 and PC2, indicating it as a characteristic compound for the effect of storage time and temperature for roots.

Different preharvest and postharvest factors, including storage temperature, have been reported to significantly affect phenolic degradation or stability [13]. A decrease in anthocyanins in red radish has been observed during storage (both in light and dark conditions) [32], while decreasing storage temperatures was reported to reduce their degradation. As mentioned before, at later storage times, the plant tissue freezes and though biological activity may stop, chemical changes can still occur that will be reflected in the metabolomic profile. In some cases cold storage has been reported to improve phenolics related quality, for example of anthocyanins and hydroxycinnamic acid derivatives [13]. An accumulation of phenylpropanoids in *Arabidopsis* at low temperature has also been reported [21]. This is may be because increased levels of phenylpropanoids could protect cells against frost-induced oxidative stress by scavenging hydrogen peroxide diffusing across membranes [31]. Low temperature acclimation includes the induction of sucrose and organic acids in plants, thus increasing the plant cell tolerance against dehydration and freezing injury [33]. The increase of organic acids and amino acids during later storage shows the selectivity and shift of plant metabolomic pathways, to those most suited for its survival and may also result in the loss of glucose and secondary metabolites. [19].

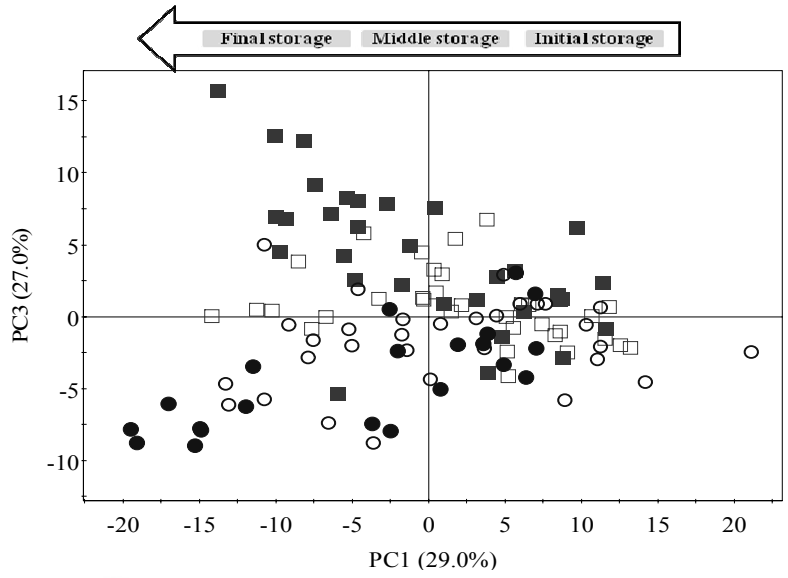

Fig 2 – A

Glucose, fumaric acid, feruloyl-, 5-hydroxyferuloyl-, sinapoyl-, coummaroyl-, and caffeoyl – malate, kaemferol, quercetin.

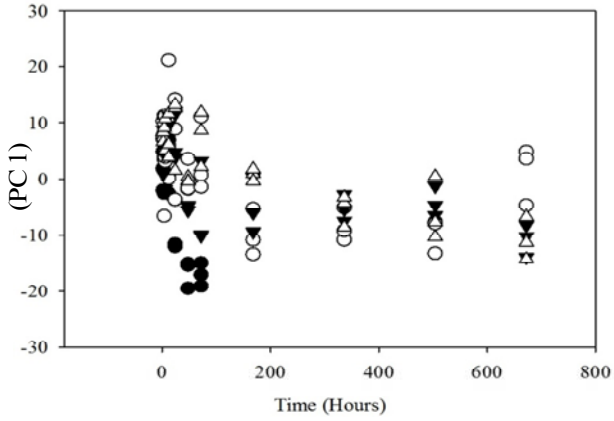

Fig 2 – B

Glucobrassicin, neoglucobrassicin, alanine, valine, threonine, GABA,

Adenine, glucose, alanine, threonine, GABA,

Glucobrassicin, neoglucobrassicin, valine, glutamine, glutamate, succrose, fumaric acid, feruloyl-, 5-hydroxyferuloyl-, sinapoyl-, coummaroyl-, and caffeoyl – malate, kaemferol, quercetin.

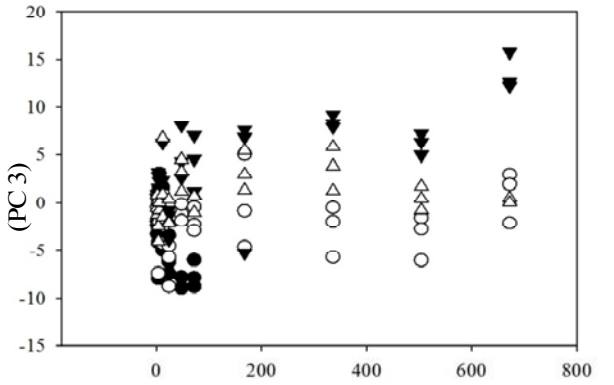

Fig 2 – C

Fig. 2: Score plot (PC1 vs PC3) of PCA for radish aerial parts (Fig. 2 – A), based on the whole range of ^{1}H-NMR signals (0.4 – 10.0); samples stored at room temperature (20 °C, ●), 4 °C (○) –20 °C (■) and –80 °C (ϒ); PC1 trends (Fig. 2 – B) and PC3 value (Figure 2 – C) throughout all the time points evaluated in this study. Initial storage (for room temperature; 2 – 6 H, other temperatures; 2 – 24 H); Middle storage (for room temperature; 12 – 24 H, other temperatures; 48 – 336 H); Final storage (for room temperature; 48 – 72 H, other temperatures; 504 – 672 H).

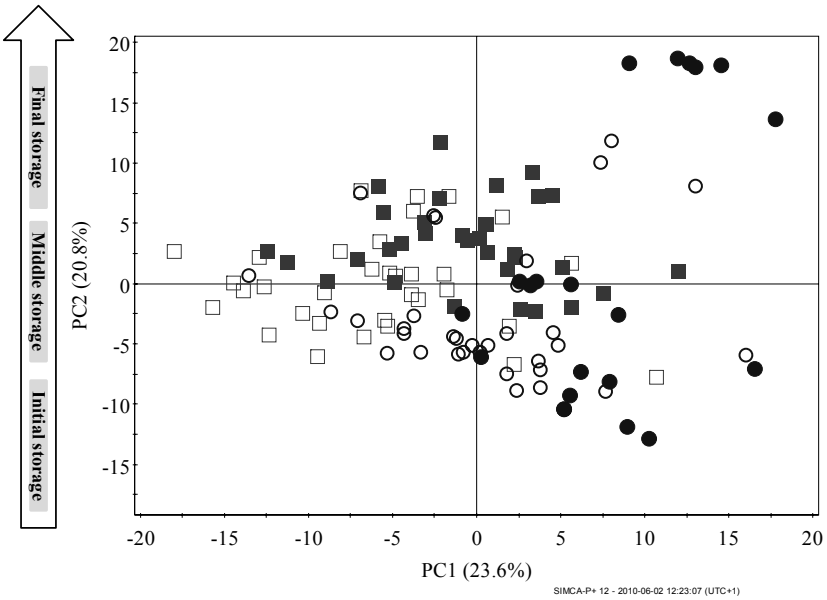

Fig 3 – A

Adenine, alanine, threonine, GABA, Glucobrassicin, neoglucobrassicin, valine, succrose, fumaric acid, feruloyl-, 5-hydroxyferuloyl-, sinapoyl-, coummaroyl-, and caffeoyl – malate, kaemferol, quercetin.

Glucose

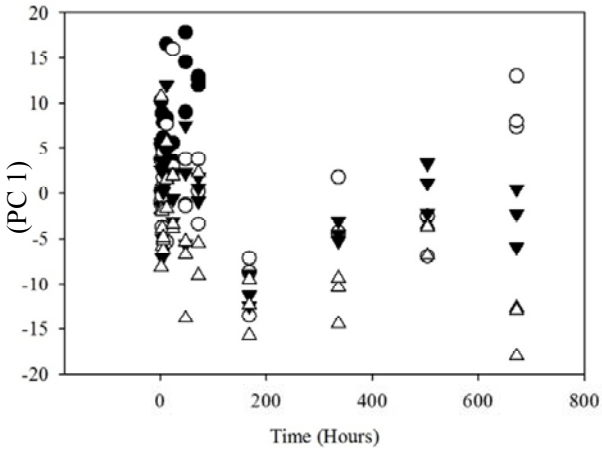

Fig 3 – B

Alanine, threonine, GABA, Glucobrassicin, neoglucobrassicin, valine, succrose, fumaric acid, feruloyl-, 5-hydroxyferuloyl-, coummaroyl-, and caffeoyl – malate, kaemferol, quercetin.

Glucose, adenine

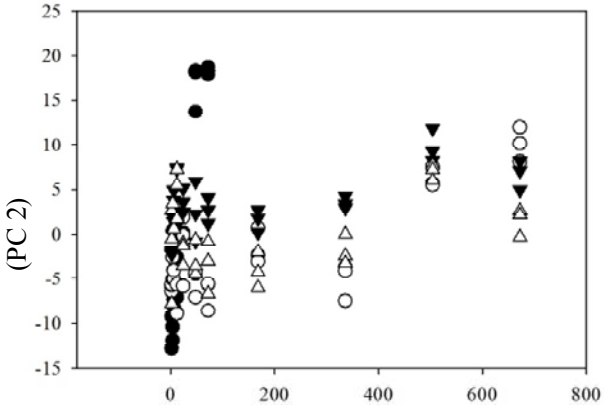

Fig 3 – C

Fig. 3: Score plot (PC1 vs PC3) of PCA for radish roots (Fig. 3 – A), based on the whole range of ^{1}H-NMR signals (0.4 – 10.0); samples stored at room temperature (20 ^{o}C, ●), 4 ^{o}C (○) –20 ^{o}C (■) and –80 ^{o}C (ϒ); PC1 trends(Fig. 2 – B) and PC3 value(Fig. 2 – C) throughout all the time points evaluated in this study.

Considering the results of NMR based metabolomic characterization coupled with multivariate data analysis, the samples stored at low temperature were found to be the closest to controls. By visual assessment for leaf and root color, loss of freshness and appeal, the quality of radish stored at 4 ^{o}C appeared to be of acceptable quality, even until 28 days of storage, but samples stored at room temperature were not acceptable after three days of storage. Samples stored at –20 ^{o}C and –80 ^{o}C looked fresh but after thawing, radish roots and leaves were not acceptable for consumption. Still –80 ^{o}C temperature should be considered as promising storage temperature of biological samples as least changes in phytochemical behavior is observed in this condition.

Experimental

Preparation of Plant Material

Seeds of *Raphanus sativus* (red radish) were sown in pots having soil medium, kept in cold room (4 °C) for 2 days and after that transported to a green house, having 16: 8 h, light: dark conditions. After 6 days of growth, the individual seedlings were transferred to separate pots and watered daily.

Fresh and healthy plants were harvested (at 8 weeks of growth) and washed thoroughly by using de-ionized water and kept in open air at room temperature for 30 minutes to remove surface water from the plant. The aerial parts (leaves and petioles) and roots were kept intact during storage.

Storage and Sample Collection

Plants were stored at four different temperatures [20 ^{o}C (room temperature), 4 ^{o}C, –20 ^{o}C, and –80 ^{o}C] in open plastic bags in the dark. Samples from each treatment were collected after 2 h, 4 h, 6 h, 12 h, 24 h (1 d), 48 h (2 d), 72 h (3 d), 168 h (7 d), 336 h (14 d), 504 h (21 d) and 672 h (28 days). After 3 days, plants stored at room temperature started decaying so further sampling was impossible. Each analysis was performed in triplicate, using one plant for each analysis. Leaves were separated from roots and immediately frozen in liquid nitrogen, ground and freeze-dried at the end, to obtain a fine powder.

Sample Extraction and NMR Measurement

Fifty mg of freeze- dried material was transferred to a microtube (2 ml) to which 1.5 ml of 50% methanol-d_4 in D_2O (KH_2PO_4 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. An aliquot of 800 ml of the supernatant was transferred to a 5 mm NMR tube. NMR measurements were done as mentioned in our previous studies [34].

Data Analysis

The ^{1}H-NMR spectra were automatically reduced to ASCII (v. 3.7, Bruker Biospin). Spectral intensities were scaled to trimethylsilyl propionic acid sodium salt (TMSP) and reduced to integrated regions of equal width (0.04) corresponding to δ 0.4 – δ 10.0. The data were normalized 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) was performed with the SIMCA-P software (v. 12.0, Umetrics, Umeå, Sweden) by using the unit variance (UV) scaling method.

Conclusion

Multivariate data analysis method is a potential method for the detection of biomarkers associated to storage at different times and temperatures. Phenolics, carbohydrates, glucosinolates and amino acids were found to be discriminating metabolites for the storage time and temperature in this study, proving the potential of PCA as a promising tool for the evaluation of post harvest metabolomic changes in vegetables.

Radish roots show less metabolomic changes as compared with leaves when evaluated by PCA. If focusing only on the effect of temperature, the least metabolomic changes were observed in the samples stored at –80 ^{o}C, so comparatively this would be the best condition to preserve research samples. It should be kept in consideration that metabolomic changes at – 80 ^{o}C (Fig. 2 and 3) should not be overlooked during research. Furthermore, the post-storage physical quality of radish (stored at – 20 ^{o}C and – 80 ^{o}C) is also not acceptable as thawing destroys structure as well as massive metabolomic changes may occur. While for quality attributes as for consumption purposes storage at 4 ^{o}C is the most preferred one due to metabolomic and physical characteristics as compared to other temperatures

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Brassicaceae vegetables offer a good base for the development of healthy food; however vegetable storage plays a critical role in the

preservation of these compounds so that further research is needed to study these compounds in relation to their breakdown products.

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References

1. E. Ciska, J. Honke and H. Kozlowska, *Journal of Agricultural and Food Chemistry*, **56**, 9087 (2008).
2. T. S. Kujala, J. M. Loponen, K. D. Klika and K. Pihlaja, *Journal of Agricultural and Food Chemistry*, **48**, 5338 (2000).
3. L. Zhao liang, L. Li-wang, L. Xiao-yan, G. Yi-qin, H. Xi-lin, Z. Xian-wen, Y. Jin-lan and W. Long-zhi, *Agricultural Sciences in China*, **7**, 823 (2008).
4. S. B. Chattopadhyay, P. K. Sahu, U. Thapa and K. M. Niranjan, *Journal of Vegetable Science*, **12**, 89 (2007).
5. N. Bellostas, P. Kachlicki, J. C. Sorensen, and H. Sorensen, *Scientia Horticulturae*, **114**, 234 (2007).
6. J. Singh, A. K. Upadhyay, K. Prasad, A. Bahadur and M. Rai, *Journal of Food Composition and Analysis*, **20**, 106 (2007).
7. F. A. Ayaz, R. H. Glew, M. Millson, H. S. Huang, L. T. Chuang, C. Sanz and S. Hayirlioglu-Ayaz, *Food Chemistry*, **96**, 572 (2006).
8. C. Martínez-Villaluenga, J. Frias, P. Gulewicz, K. Gulewicz and C. Vidal-Valverde, *Food and Chemical Toxicology*, **46**, 1635 (2008).
9. S. G. Hegde, J. D. Nason, J. M. Clegg and N. C. Ellstrand, *Evolution*, **60**, 1187 (2006).
10. P. Jing, S. J. Zhao, S. Y. Ruan, Z. H. Xie, Y. Dong and L. Yu, *Food Chemistry*, **133**, 1569 (2012).
11. I. S. Curtis, *Trends in Plant Science*, **8**, 305 (2003).
12. D. K. Asami, Y. J. Hong, D. M. Barrett and A. E. Mitchell, *Journal of the Science of Food and Agriculture*, **83**, 56 (2003).
13. F. Tomas-Barberan and J. C. Espin, *Journal of the Science of Food and Agriculture*, **81**, 853 (2001).
14. S. S. Sablani, *Drying Technology,* **24**, 123 (2006).
15. K. S. Bahceci, A. Serpen, V. Gokmen and J. Acar, *Journal of Food Engineering*, **66**, 187 (2005).
16. R. K. Toor and G. P. Savage, *Food Chemistry*, **99**, 724 (2006).
17. A. Podsedek, *Lwt-Food Science and Technology*, **40**, 1 (2007).
18. L. W. Sumner, P. Mendes and R. A. Dixon, *Phytochemistry*, **62**, 817 (2003).
19. J. S. del Aguila, F. F. Sasaki, L. S. Heiffig, E. M. M. Ortega, A. P. Jacomino and R. A. Kluge, *Postharvest Biology and Technology*, **40**, 149 (2006).
20. M. Ali, Atta-ur-Rehaman, I. Bayoumi and M. Jahangir, *Journal of Bio-Molecular Sciences*, **1**, 1 (2013).
21. M. Mattana, E. Biazzi, R. Consonni, F. Locatelli, C. Vannini, S. Provera and I. Coraggio, *Physiologia Plantarum*, **125**, 212 (2005).
22. O. Fiehn, J. Kopka, P. Dormann, T. Altmann, R. N. Trethewey and A. L. Willmitzer, *Nature Biotechnology*, **18**, 1157 (2000).
23. M. Ziegler, M. Engel, G. Welzl and M. Schloter, *Journal of Microbiological Methods*, **94**, 30 (2013).
24. M. Jahangir, H. K. Kim, Y. H. Choi and R. Verpoorte, *Food Chemistry*, **107**, 362 (2008).
25. I. B. Abdel-Farid, H. K. Kim, Y. H. Choi and R. Verpoorte, *Journal of Agricultural and Food Chemistry*, **55**, 7936 (2007).
26. M. Jahangir, I. B. Abdel-Farid, Y. H. Choi and R. Verpoorte, *Journal of Plant Physiology*, **165**, 1429 (2008).
27. I. Kaplan, R. Halitschke, A. Kessler, B. J. Rehill, S. Sardanelli and R. F. Denno, *Ecology Letters*, **11**, 841 (2008).
28. Y. S. Liang, Y. H. Choi, H. K. Kim, H. J. M. Linthorst and R. Verpoorte, *Phytochemistry*, **67**, 2503 (2006).
29. J. R. Eason, D. Ryan, B. Page, L. Watson and S. A. Coupe, *Postharvest Biology and Technology*, **43**, 358 (2007).
30. G. A. King and S. C. Morris, *Journal of the American Society for Horticultural Science*, **119**, 1000 (1994).
31. S. J. Kim and G. Ishii, *Journal of the Science of Food and Agriculture*, **87**, 966 (2007).
32. P. Bednarek, B. Schneider, A. Svatos, N. J. Oldham and K. Hahlbrock, *Plant Physiology*, **138**, 1058 (2005).
33. J. Danyluk, A. Perron, M. Houde, A. Limin, B. Fowler, N. Benhamou and F. Sarhan, *Plant Cell*, **10**, 623 (1998).
34. H. K. Kim, Y. H. Choi and R. Verpoorte, *Nature Protocols*, **5**, 536 (2010).