Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. *81* (6) 623–632 (2016)
JSCS–4872

UDC 582.688.4:577.164.1/.2+54–145.2+
621.565.92
Original scientific paper

Changes in the content of water-soluble vitamins in *Actinidia chinensis* during cold storage

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(Received 28 October, revised 18 December 2015, accepted 29 January 2016)

Abstract: The effects of cold storage on nine water-soluble vitamins in seven cultivars of *Actinidia chinensis* (kiwifruit) were assessed using high-performance liquid chromatography. Samples were collected at three time points during cold storage: one day, 30 days, and when edible. It was found that the vitamin C content in most cultivars increased with cold storage time, but there was no consistent increasing or decreasing trend for the other water-soluble vitamins across the cultivars during storage. After one day of cold storage, vitamins B_1 and B_2 were the most prevalent vitamins in the control (wild) fruit, while vitamins B_5 and B_6 were most prevalent in the Hongyang and Qihong cultivars. However, B_{12} was the most prevalent vitamin in the Qihong cultivar after 30 days of cold storage. Vitamins B_3, B_7, B_9, and C were detected at the edible time point in the Huayou, Hongyang and Jingnong-2 cultivars and in the control fruit. The vitamin contents varied significantly among kiwifruit cultivars following different durations of cold storage. Out of the three durations tested, 30 days in cold storage was the most suitable for the absorption of water-soluble vitamins by *A. chinensis*.

Keywords: vitamin B; kiwifruit; cultivar; storage; vitamin C.

INTRODUCTION

Water-soluble vitamins are found in very small amounts in edible foods, but they play an essential role in the metabolism of the human body. Their deficiency leads to a variety of clinical abnormalities that range from anemia to growth retardation and neurological disorders.[1] Vegetables and fruits are the major dietary sources for water-soluble vitamins in humans, and much research has been conducted on the vitamin content of various vegetables and fruits, such as six

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doi: 10.2298/JSC151028012Z

date palm cultivars, green leafy vegetables, *Rhodiola imbricata* root, black garlic, and okra.[2–6] Changes to the water-soluble vitamin content of black garlic and six date palm cultivars were registered following thermal processing or vegetable storage.[2,3,5]

Kiwifruits have a unique flavor and are rich in vitamin C, dietary fiber, and a variety of mineral nutrients, making them popular with consumers around the world. The most commonly cultivated types are *Actinidia chinensis* and *A. deliciosa*. *A. chinensis* kiwifruits are endemic to China, and the Chinese public generally prefers *A. chinensis* to *A. deliciosa*. Although it was found that kiwifruit might contain up to nine water-soluble vitamins, little research has been performed to determine which factors influence the vitamin content and, in particular, what the ideal cold-storage period is for vitamin uptake.[7]

Water-soluble vitamins can be measured with either liquid chromatography–mass spectrometry (LC–MS) or high-performance liquid chromatography (HPLC), though HPLC tends to be the more cost-effective method.[2,5,7–16] Thus, in this study, the effects of cold storage on the water-soluble vitamin content of 7 *A. chinensis* cultivars were assessed using HPLC. It is hoped that this research may provide a guide for how to maximize the uptake of water-soluble vitamins by kiwifruit.

EXPERIMENTAL

Plant materials

Seven kiwifruit cultivars were selected for this study: *A. chinensis* wild kiwifruit (here after referred to as control), Wudang-1, Hongyang, Qihong, Huayou, Jingnong-2, and Cuiyu. The control (wild) kiwifruit were picked on the Qinling Mountains in Shaanxi province. Wudang-1 kiwifruit were obtained from the Institute of Economic Crop Research Center in Shiyan City (32°65′N, 110°79′E), Hubei, China. Hongyang and Qihong kiwifruit were retrieved from two well-managed orchards in Meixian (34°29′N, 107°76′E), Shaanxi, China. Huayou, Jingnong-2 and Cuiyu were acquired from the Kiwifruit Experiment Station of the Northwest Agricultural & Forestry University in Shaanxi province. All plant material were picked when the total soluble solids were 6.5–9.5 % of the harvest term and immediately transported to the laboratory, where they were rapidly pre-cooled before being stored under refrigerated conditions.[17] As some cultivars took over two months in cold storage (1±0.5 °C) to soften, samples were collected from all cultivars at the picking period (Day 1), after 30 days of storage (Day 30), and then when they reached the edible period (EP) for assessment of the content of water-soluble vitamins. The storage times were as follows: control, 56 days; Wudang-1, 61 days; Qihong, 61 days; Hongyang, 62 days; Cuiyu, 81 days; Huayou, 71 days and Jingnong-2, 61 days.

Reagents

All chemicals were of analytical reagent grade, and Milli-Q water was distilled and filtered through a 0.22-μm membrane filter. Thiamine hydrochloride (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin B_3), pantothenic acid (vitamin B_5), pyridoxine hydrochloride (vitamin B_6), D-biotin (vitamin B_7), folic acid (vitamin B_9), cyanocobalamin (vitamin B_{12}), and ascorbic acid (vitamin C) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Sodium hexanesulfonate and phosphoric acid were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjing, China). HPLC-grade methanol was purchased from ThermoFisher Company (Waltham, USA).

Sample preparation

Samples (10 g) were prepared from the pulp of five kiwifruits for each cultivar and were then homogenized. Sample extracts were prepared with ultrasonic assistance in 30 mL of HCl (0.005 M) for 30 min at 30 °C. All extracts were centrifuged at 10,000×g for 5 min at 4 °C and then filtered through a 0.22-μm nylon membrane. During all these steps, the samples were protected from light. Three independent replicates were prepared for each sample.

*Determination of firmness and total soluble solids (*TSS*)*

Firmness was measured in five kiwifruits, and the measurements were repeated three times. A TA-XT2i texture analyzer (Stable Microsystems, Godalming, UK) was used to determine firmness using 8 mm diameter samples. The fruits were peeled at opposite side and then the firmness was determined for the peeled position.[18] The *TSS* was assessed by juicing five kiwifruits and then using a PAL-1 refractometer (Atago, Japan).

HPLC analysis

A Shimadzu SCL-10AVP HPLC system was used. The mobile phase consisted of (A) methanol and (C) 0.005 M sodium hexanesulfonate (phosphoric acid was used to adjust the pH to 3.0). A C18 (YMC-Pack ODS-A, 4.6×250 mm, 5 μm) column was used for the determination of water-soluble vitamins. In all cases, the flow rate was 0.7 mL min^{-1} using isocratic elution (A:C = 3:7). The column temperature was set at 25 °C, and injection volume was 10 μL. Dual wavelength recording was used detect the vitamins simultaneously under the same conditions: B_5 and B_7 were detected at 210 nm, while the other seven water-soluble vitamins were detected at 270nm.[19] HPLC chromatograms are shown in Supplementary material to this paper (Fig. S-1)

Statistical analysis

IBM SPSS Statistics software v.20 and SigmaPlot 12.0 were employed for data elaboration and statistical analysis. All analyses were run in three duplicates with five kiwifruits of each cultivar used in each duplicate and the results are expressed as means ± standard deviation (*SD*). One-way analysis of variance (ANOVA) was employed to assess the differences in the vitamin contents for different storage times. Differences (Duncan) tests were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Determination of firmness and total soluble solids

Firmness is usually used as a proxy for the degree of maturity of kiwifruit, while the total soluble solids (*TSS*) is an important indicator of kiwifruit quality.[17] When kiwifruit picked period follow $6.5 \leq TSS \leq 7.5$, and the firmness ≤1 N mm^{-1}, there was no need to store. The first period followed $6.5 \leq TSS \leq 7.5$, the second was stored 30 days, and the last period was when the firmness was ≤1 N mm^{-1} (Table I). When the sampled kiwifruit were separated from the vine, their firmness decreased, while *TSS* increased in all cultivars with the duration of storage (Table I).

TABLE I. Changes in firmness and total soluble solids (*TSS*) during cold storage of kiwifruit; each value represents the mean ± standard deviation. Different letters (a–c) within a row indicate significant differences at $p < 0.05$ over the three storage time points for each cultivar

Cultivar	Firmness, N mm^{-1}			Total soluble solids, %		
	Day 1	Day 30	Edible period	Day 1	Day 30	Edible period
Control	6.47±0.07^a	1.58±0.06^b	0.36±0.02^c	6.46±0.05^c	12.9±0.13^a	12.0±0.11^b
Wudang-1	6.29±0.06^a	3.96±0.05^b	0.89±0.02^c	6.52±0.06^c	11.8±0.08^b	13.6±0.15^a
Huayou	6.23±0.05^a	5.30±0.07^b	0.96±0.07^c	6.66±0.07^c	9.7±0.12^b	12.5±0.12^a
Cuiyu	7.37±0.08^a	7.00±0.05^a	0.71±0.03^b	7.46±0.15^c	11.2±0.08^b	16.5±0.12^a
Jingnong-2	4.77±0.04^a	4.38±0.04^b	0.44±0.01^c	9.40±0.11^c	14.1±0.11^b	17.2±0.10^a
Hongyang	5.33±0.06^a	5.28±0.07^a	0.75±0.02^b	6.86±0.16^c	11.0±0.10^b	17.2±0.10^a
Qihong	6.59±0.08^a	4.37±0.09^b	0.97±0.02^c	7.12±0.15^c	15.3±0.13^b	20.0±0.12^a

Changes in B_1, B_2, B_3, and B_5 in cold storage

For each of the nine vitamins assessed, the water-soluble vitamin content ranged from undetectable to more than 2500 μg g^{-1} fresh weight (FW). Out of the nine water-soluble vitamins previously reported in kiwifruit, only B_9, B_{12} and vitamin C were detected in all cultivars across all three periods. In most cultivars, these three vitamins became more prevalent in the fruit over time.

Vitamin B_1 was not detected on Day 1 in the Wudang-1 samples, while it was only detected on Day 30 in the Cuiyu samples and only on Day 1 in the Qihong fruit (Fig. 1A). In the Jingnong-2 cultivar, the vitamin B_1 content remained constant over the three time points, indicating that cold storage had no effect on the B_1 content in this cultivar. Previous studies found that the B_1 content increased over time, a phenomenon that was observed in the present study for the Wudang-1 and Hongyang cultivars. In contrast, it was found that the B_1 content in the control and Huayou fruit decreased with storage.[3]

Vitamin B_2 can be destroyed by light and heat; therefore, rapid extraction in a dark room was used to avoid decomposition. The vitamin B_2 content in all cultivars across the three points was found to be no higher than 0.1 μg g^{-1} FW (Fig.1B), except for in the Control cultivar on Day 1. The Vitamin B_2 content increased in Huayou fruit over time, perhaps due to microbial growth.[20] The B_2 content decreased in the control and Hongyang cultivars, while the others exhibited no significant differences across the three time points despite a low vitamin B_2 content. This may be because B_2 participates in physiological metabolism in storage.[21]

The control, Wudang-1 and Qihong cultivars had the highest vitamin B_3 content on Day 30, while the Huayou, Cuiyu, and Jingnong-2 cultivars experienced an increase in B_3 content across the three time points (Fig.1 C). The maximum B_3 content (over 0.2 μg g^{-1} FW) was found in the Huayou fruit at the edible period.

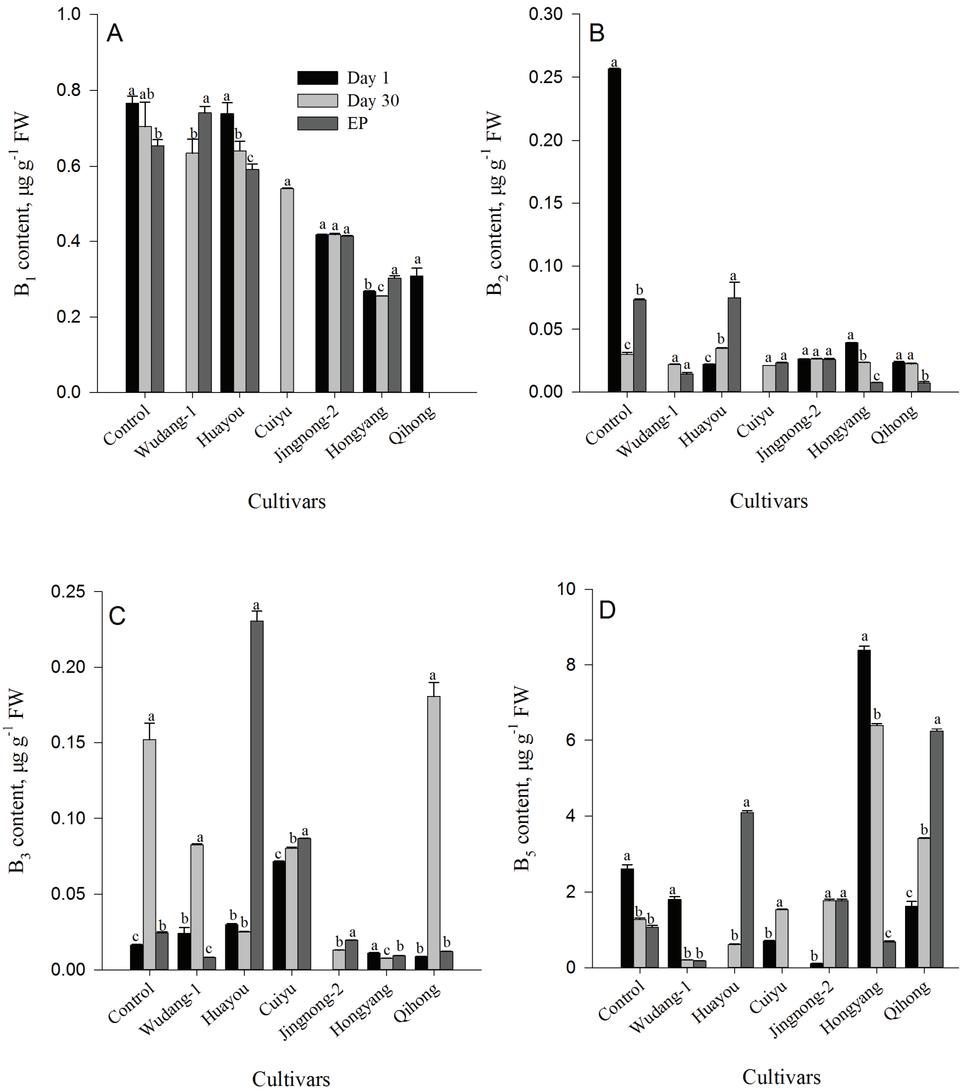

Fig. 1. Changes in the B_1 (A), B_2 (B), B_3 (C) and B_5 (D) contents of *A. chinensis* during cold storage.

Interestingly, the vitamin B_5 content increased in three cultivars over time and decreased in three cultivars over time. Jingnong-2 was found to have the highest B_5 content with more than 8 μg g^{-1} FW on Day 30 (Fig. 1D).

Changes of B_6, B_7, B_9, B_{12} and vitamin C in cold storage

Of the seven cultivars, the vitamin B_6 content was not detectable in only two cultivars: Huayou on Day 1 and Cuiyu on Day 1 and at the EP (Fig. 2A). The B_6

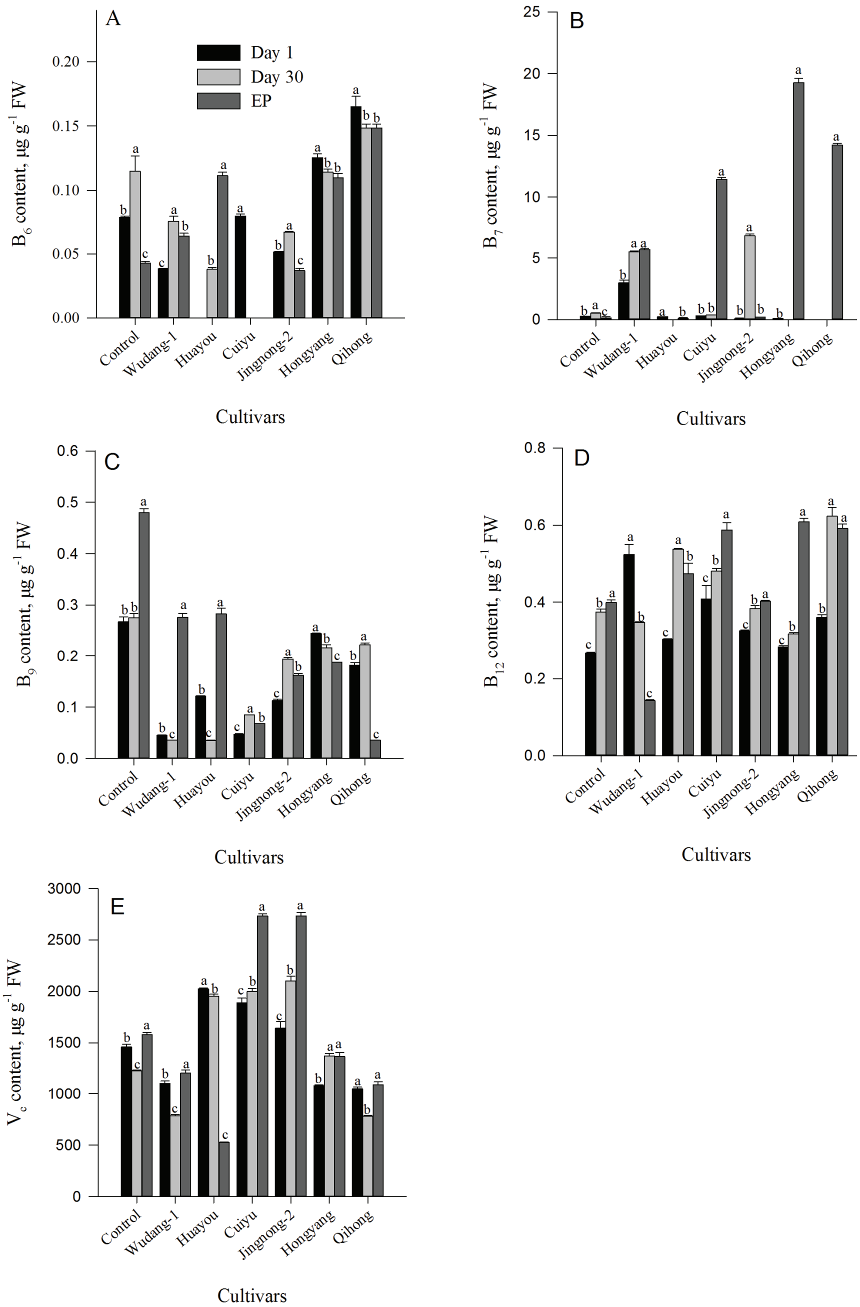

Fig. 2. Changes in the B_6 (A), B_7 (B), B_9 (C), B_{12} (D) and vitamin C (E) contents of *A. chinensis* during cold storage. Each value represents the mean ± standard deviation. Different letters (a–c) within a row indicate significant differences at the $p < 0.05$ level over the three storage time points for each cultivar.

content in some cultivars was not detected, which could be attributed to the hydroxylation of B_6 in the presence of vitamin C. In general, except for Huayou, the B_6 contents of the other six cultivars were lower at the edible time point than at Day 30. Similar results were previously found with the storage of green leafy vegetables.[3]

Biotin (vitamin B_7) is involved in amino acid catabolism, gluconeogenesis, and fatty acid synthesis. The vitamin B_7 content of four cultivars increased with the duration of cold storage. Jingnong-2, on the other hand, had the highest B_7 content on Day 30, while the other cultivars had their lowest B_7 contents at that time (Fig. 2B). The highest B_7 content registered in this study appeared in the Hongyang fruit and was nearly 20 μg g^{-1} FW.

Rapid increases in the vitamin B_9 content were found from Day 30 to the edible time point in the control, Wudang-1 and Huayou kiwifruit (Fig. 2C). In the same period, a rapid decrease in the Qihong cultivar was found. The highest B_9 content (nearly 0.5 μg g^{-1} FW) was found in the control fruit at the EP.

For most cultivars, the vitamin B_{12} content increased with increasing duration of cold storage. The exceptions were Wudang-1, where B12 decreased over time, and Huayou and Qihong, where Day 30 exhibited the highest B_{12} content (Fig. 2D).

Vitamin C was found to be abundant in kiwifruit, although no regular pattern was found across the seven cultivars with cold storage. For example, for the control, Wudang-1 and Qihong cultivars, the lowest vitamin C content was detected on Day 30, while the content decreased over time for the Huayou fruit (Fig. 2E). For the other cultivars, the vitamin C content increased with increasing duration of storage.

For all the studied *A. chinensis* cultivars, Day 30 was found to be the time point most suitable for absorbing water-soluble vitamins, as most of them could be detected in the seven cultivars at this time (Figs. 1 and 2). Although all water-soluble vitamins were detected at all three time points in the control (wild) kiwifruit, these fruit had the lowest *TSS* content (Table I), making the fruit less palatable for human consumption. Previous studies found a significant depletion of vitamin C in fruit juice with cold storage.[22] In the present study, however, the vitamin C content increased for many cultivars. This phenomenon was also found in pea leaves, perhaps due to the bound form of vitamin C being released at different times.[3] Similarly, the effects of cold storage on the other water-soluble vitamins were not similar to those seen in fruit juice; this perhaps be due to water-soluble vitamins in living plant materials having some activity.[3,22]

The Hongyang cultivar is one of the most popular cultivars and of high economic importance in northwest China, and it was found to be rich in all water-soluble vitamins (Figs. 1 and 2). It is therefore the most suitable cultivar of the seven cultivars for absorbing water-soluble vitamins. Water-soluble vitamins not detectable in some cultivars at some time points, which corresponded to LC–MS results.[10] The B_1, B_2, B_5 and B_6 contents in some cultivars increased over time. Similar results were obtained during vegetables storage, indicating that the levels of these three vitamins reflect not only degradation but also synthesis.[3]

Some previous studies used LC–MS to detect water-soluble vitamins in green and yellow kiwifruit.[7] In the present study, the contents of B_6 and B_9 found were lower and that of B_7 were higher than the values found in previous studies, perhaps due to differences in varieties studied or cultivation environments.[7] Interestingly, the B_7 contents found in the two red kiwifruit (Hongyang and Qihong cultivars) were higher than those of green kiwi, red kiwifruit may have more efficient synthetic pathways in regards to this vitamin.[7] In green leafy vegetables, the contents of several water-soluble vitamins change during storage, but B_7 was not one of these. In fact, sometimes B_7 could not be detected at all for it is often in the bound form and not often in the free form.[3]

Vitamin B_9 was detected in all kiwifruit cultivars across the three time points. This is in contrast to stored vegetables, which do not have abundant vitamin B_9.[3] Similarly, stored vegetables have very low vitamin B_{12} contents, while *A. chinensis* cultivars contain considerable levels of B_{12}.[3]

CONCLUSIONS

The contents of nine water-soluble vitamins changed over time in *Actinidia chinensis*. Different cultivars exhibited different trends for different vitamins. For example, vitamin C in most cultivars increased with storage, while the contents of the other vitamins showed no similar change trends. Some vitamins were undetectable in some cultivars at one or two time points. In general, more water-soluble vitamins could be detected following storage for 30 days, indicating this is the optimal duration of cold storage for the uptake of water-soluble vitamins.

SUPPLEMENTARY MATERIAL

HPLC chromatograms of a standard mixture for the studied water-soluble vitamins are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgements. This work was supported by the earmarked fund for Modern Agro-industry Technology Research System of China. We would like to thank Dr. Jiangtao Suo for material assistance.

И З В О Д

ПРОМЕНА САДРЖАЈА ВИТАМИНА РАСТВОРНИХ У ВОДИ У БИЉЦИ *Actinidia chinensis* ТОКОМ ЧУВАЊА НА ХЛАДНОМ

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Испитана је промена у садржају девет витамина растворних у води седам сорти *Actinidia chinensis* (киви) током чувања на хладном, применом методе HPLC. Сакупљена су три узорка током чувања: првог дана, после 30 дана и у тренутку сазревања за јело. Садржај витамина Ц је порастао у већини сорти током чувања, али за друге витамине није нађена правилност у промени. Након једног дана чувања на хладном, витамини $Б_1$ и $Б_2$ су били доминантни у контролном (дивљем) плоду, док су витамини $Б_5$ и $Б_6$ били доминантни у сортама *Hongyang* и *Qihong*. После 30 дана чувања, витамин $Б_{12}$ је постао главни у сорти *Qihong*. Витамини $Б_3$, $Б_7$, $Б_9$ и Ц су детектовани тек у зрелим плодовима сорти *Huayou*, *Hongyang*, *Jingnong-2* и у контролној сорти. Садржај витамина је јако варирао између сорти кивија у различитим периодима чувања. Упоређивањем три временска периода чувања, закључено је да је, након 30 дана чувања, најповољнија комбинација витамина *A. chinensis* за апсорпцију.

(Примљено 28. октобра, ревидирано 18. децембра 2015, прихваћено 29. јануара 2016)

REFERENCES

1. B. Klejdus, J. Petrlová, D. Potěšil, V. Adam, R. Mikelová, J. Vacek, R. Kizek, V. Kubáň, *Anal. Chim. Acta* **520** (2004) 57
2. J. Aslam, S. H. Khan, S. A. Khan, *J. Saudi Chem. Soc*. **17** (2013) 9
3. J. Santos, J. A. Mendiola, M. B. P. P. Oliveira, E. Ibanez, M. Herrero, *J. Chromatogr. A* **1261** (2012) 179
4. A. B. Tayade, P. Dhar, J. Kumar, M. Sharma, O. P. Chaurasia, R. B. Srivastava, *Anal. Chim. Acta* **789** (2013) 65
5. J. S. Kim, O. J. Kang, O. C. Gweon, *Food Sci. Biotechnol*. **22** (2013) 283
6. R. Sami, Y. Li, B. K. Qi, S. N. Wang, Q. Z. Zhang, F. F. Han, Y. Ma, J. Jing, L. Z. Jiang, *J. Chem.* (2014), Article ID 831357
7. A. Gentili, F. Caretti, G. D'Ascenzo, S. Marchese, D. Perret, D. Di Corcia, L. M. Rocca, *Rapid Commun. Mass Spectrom*. **22** (2008) 2029
8. P. Chen, W. R. Wolf, *Anal. Bioanal. Chem*. **387** (2007) 2441
9. R. Engel, L. Abranko, E. Stefanovits-Banyai, P. Fodor, *Acta Aliment.* **39** (2010) 48
10. R. Ekinci, *Food Chem*. **90** (2005) 127
11. O. Heudi, T. Kilinc, P. Fontannaz, *J. Chromatogr. A* **1070** (2005) 49
12. P. Moreno, V. Salvado, *J. Chromatogr. A* **870** (2000) 207
13. E. Paredes, M. Prats, S. E. Maestre, J. L. Todoli, *Food Anal. Methods* **5** (2012) 897
14. A. Lebiedzinska, M. L. Marszall, J. Kuta, P. Szefer, *J. Chromatogr. A* **1173** (2007) 71
15. R. Amižić, J. Brborić, O. Čudina, S. Vladimirov, *J. Serb. Chem. Soc*. **70** (2005) 1229
16. A. Gliszczyńska-Świgło, I. Rybicka, *Food Anal. Method*. **8** (2014) 139
17. B. C. Strik, *Growing Kiwifruit*, Pacific Northwest Extension, Oregon State University, 2004, p. 507
18. D. Valero, J. M. Valverde, D. Martínez-Romero, F. Guillén, S. Castillo, M. Serrano, *Postharvest Biol. Technol*. **41** (2006) 317

19. S. Vidović, B. Stojanović, J. Veljković, L. Pražić-Arsic, G. Roglić, D. Manojlović, *J. Chromatogr. A* **1202** (2008) 155
20. M. C. Sanchez-Mata, M. Camara, C. Diez-Marques, *Food Chem*. **80** (2003) 309
21. F. Depeint, W. R. Bruce, N. Shangari, R. Mehta, P. J. O′Brien, *Chem. Biol. Interact*. **163** (2006) 94
22. L. Salvia-Trujillo, M. Morales-de la Pena, A. Rojas-Grau, O. Martin-Belloso, *J. Agric. Food Chem*. **59** (2011) 10034.