

CHANGES OF BIOACTIVE COMPOUNDS CONTENT OF KIWIBERRY DURING STORAGE

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Summary. The objective of the research study was to determine of on biochemical properties and bioactive compounds content changes of kiwiberry (*Actinidia arguta*) stored for up to 10 days at storage temperature (5, 15, 25°C) then kiwiberry fruits was frozen and stored until testing. This work examines the temperature and storage time of the fruit of the *Actinidia arguta* on their acidity, flavonoid content, the total content of polyphenol compounds and antioxidant activity. The results resulted in the finding that different temperatures and storage time had a statistically significant effect of the physico-chemical properties of the minikiwi fruit. The use of higher temperatures accelerated the ripening process, which resulted in a decrease in fruit acidity. Additionally, during storage at higher temperatures an increase in the content of valuable compounds, including flavonoids having a beneficial effect on human health, was noted.

Key words: storage, *Actinidia arguta*, antioxidant capacity, total phenolic content, flavonoids, carotenoids

INTRODUCTION

Maintaining very high quality of fruits during storage is of interest to food industry, especially to the fruit growing and distribution industries [Chen et al. 2015]. Therefore, during the storage there are a number of chemical and biochemical reactions which reduce their quality. The ageing of the fruit during their long-term storage results from reduced physiological activity. There is also a significant loss of usable value due to the intense transpiration process and water loss. In addition, the quality of fresh fruits deteriorate rapidly mainly due to cellular juice and as mentioned above water loss. However, storage conditions also have a huge impact on the content of bioactive compounds. The

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main problem is that they are susceptible to oxidation reactions, which can negatively affect the antioxidant capacity in fruits. Dynamic variations in chemical composition and tissue structure cause changes in chemical, physical and sensory properties [Złotek and Wójcik 2012, Duarte-Molina et al. 2016, Mannozi et al. 2018]. Discussed in this article kiwiberry belongs to climacteric fruits. Under the influence of ethylene processes of fruit ripening and softening are much faster than in normal conditions [Stefaniak et al. 2017]. Berries such as minikiwi are very susceptible to quick spoilage and very highly perishable during inappropriate storage conditions. For example, fresh blueberries have a shelf life of 1–8 weeks mainly depending on stage of their ripeness [Chen et al. 2015]. One of the main factors limiting capability of using berries is softening and loss of firmness [Ciupak and Gładyszewska 2010, Chen et al. 2015].

Softening is first of all due to the change in cell-wall carbohydrate metabolism in fruits. This leads to a decrease in the content of selected components, for example structural. Softening process is also related to reduction total water soluble pectin and the disassembly of primary cell-wall and middle lamella structures ingredients. Cell-wall modifications are mainly hemicelluloses depolymerisation and the loss of arabinose. These modifications, including degradation of the cell-wall, results from the coordinated action of hydrolytic enzymes (especially polygalacturonase as well as glycosidases and glycanases) [Chen et al. 2015]. Brillante et al. [2015] concluded that berries change their physical properties during the ripening progresses, and at the same time accumulate flavonoids in the skin. Stefaniak et al. [2017] argued that changes in kiwiberry chemical composition and their quality is not yet known.

However, it is known that Actinidia fruits contain a range of bioactive ingredients with bacteriostatic properties, antioxidants and high enzyme activity, what is their added value. In addition, it is a rich source of various phytochemicals, such as vitamins (vitamin C and B), minerals, organic acid, phenolics (especially polyphenols), flavonoids, carotenoids (especially β -carotene and lutein) and pigments (β -carotene, chlorophylls, anthocyanins). The kiwiberry has become more and more often popular not only due to taste and pleasant sensory characteristics, but also due to potential pro-health effects. High consumers acceptance appreciate kiwiberry for positive nutritional and health values [Latocha et al. 2010, Latocha 2012, Latocha et al. 2015, Bialik et al. 2017, Latocha 2017, Bialik et al. 2018].

The aim of the study was to determine of on biochemical properties and bioactive compounds content changes of kiwiberry (*Actinidia arguta*) during storage at different temperature stored (5, 15, 25°C) for up to 10 days.

MATERIAL AND METHODS

Material

Experimental material was fruits of hardy kiwi (*Actinidia arguta* Polish variety 'Weiki'). Plants grew in the commercial plantation under supervision of scientists from the Environmental Protection Department, Warsaw University of Life Sciences – SGGW, Poland.

Storage and treatment of kiwiberry fruits

Fruits at the eating maturity stage (harvested in October) were stored at three different temperatures (5, 15 and 25°C) for a period of 10 days. After a certain period of time (at one- or two-day intervals), the fruit was frozen and stored until the appropriate series of experiments. Before each experiment, the Actinidia fruits were thawed, crushed (milled) and prepared according to the assay instructions. Sample codes of experimental runs developed in accordance with the scheme presented in the table below.

Table. The scheme of two-factorial design of sample codes

Tabela. Schemat oznaczenia próbek za pomocą kodów

Run Przebieg	Storage time [days] Czas przechowywania [dni]	Storage temperature Temperatura przechowywania [°C]	Sample codes Przykład kodu
1	1	5	1_5
2	1	15	1_15
3	1	25	1_25
4	5	5	5_5
5	5	15	5_15
6	5	25	5_25
7	10	5	10_5
8	10	15	10_15
9	10	25	10_25

Analytical analysis

Dry matter

For each sample the dry matter was measured by the weight method. The mill-crushed material (2 g) was weighed into glass vessels and dried in a laboratory dryer with natural air circulation (POL-EKO SLN 115, Poland) at 70°C for 24 h. The dry matter content was calculated on the basis of weight loss during drying. The determination was made in duplicate.

Total phenolic content

The total polyphenolic content was determined using Folin-Ciocalteu method, in accordance with modified procedure presented previously by Nowacka et al. [2014] and Wiktor et al. [2016]. Extraction from the samples was carried out using an 80% ethanol solution. Samples were ground in an analytical mill (A10 Basic, IKA, Germany). About 20 ml of extraction reagent was added to 1.5 g of material, homogenized and heated on a hot plate until boiling. The solution was filtered into a flask and made up 80% ethanol to a volume of 50 ml. The extracts prepared in this way were used to determine the content of polyphenols, flavonoids and anti-radical activities. Volume of 4.92 ml of distilled water, 0.18 ml of extract and 0.3 ml of F-C reagent were added into glass tubes to determine total polyphenols content. The solution was mixed on a vortex and after 3 min 0.6 ml of 17.7% sodium carbonate solution was added, mixed and stored in the dark at room temperature for 1 h.

A blank was prepared analogously, the extract was replaced with 0.18 ml of an 80% ethanol solution. The absorbance of the solutions was measured using a UV-VIS spectrophotometer (Thermo Spectronic Helios Gamma, Thermo Fischer Scientific, USA) at 750 nm, against a blank. The results are expressed in mg of gallic acid per 100 g of dry matter. The determinations were made in three replicates for each type of material.

Total flavonoids content

To assess the flavonoid content a spectrophotometric method based on measuring the absorbance of coloured complexes of flavonoid compounds with aluminum chloride was used.

To 2 ml ethanolic extract of the sample 2 ml 2% aluminum chloride solution (in 80% ethanol) was added and mixed. After 10-minute incubation at room temperature, in the dark absorbance at 430 nm was measured against an 80% ethanolic solution with a spectrophotometer (Thermo Spectronic Helios Gamma, Thermo Fischer Scientific, USA). The determination was performed in duplicate for each of the experiment variants tested. Results are expressed in mg quercetin per 1 g of dry matter.

Antioxidant assay method

The antioxidant measure procedure was reported by Nowacka et al. [2018]. Stock solution of ABTS^{•+} radical was prepared by dissolving in 5 ml of distilled water 19.2 mg of 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate) cation-radical, 3.3 mg K₂S₂O₈. Before analysis, a radical working solution was prepared by diluting the stock solution with 80% ethyl alcohol to obtain a solution with absorbance at 734 nm at 0.680–0.720. Volume of 20, 40, 60, 80 µl ethanol extract was added to the glass tubes and 3 ml of ABTS working solution, mixed. After 6 min incubation at room temperature without light, the absorption of radical and solutions was measured at 734 nm. The scavenging of ABTS presented in Actinidia fruit's extracts was expressed as the concentration of extract required to reduce a half of free radicals (EC50).

Total carotenoids content

The total content of carotenoids was determined based on the spectrophotometric method according to the Polish standard PN-EN 12136:2000 [Nowacka and Wedzik 2016]. To 1.5 g grinded sample 20 ml of distilled water, 1 ml of Carrez I solution, 1 ml of Carrez II solution were added to precipitate high molecular weight compounds i.e. the proteins, mixed and after 2 min centrifuged (2,000 g, 5 min). Carotenoids were extracted three times with portions of 25 ml acetone. Petroleum ether (45 ml) was added to the supernatant and mixed. After phase aberration the absorbance of solutions were measured at 450 nm. The total carotenoids content was expressed in mg β-carotene per 100 g of dry matter.

Total acidity

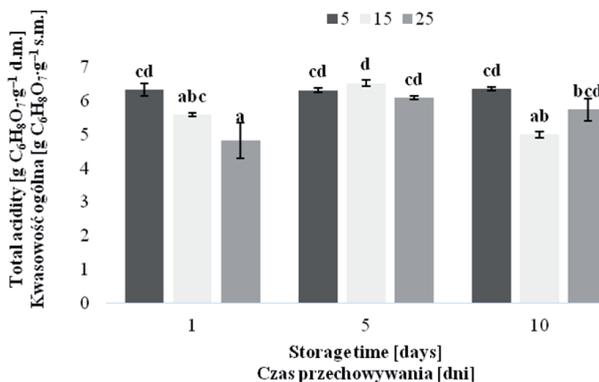
The total acidity was determined using a titration method [Cendrowski and Mitek 2012, Ochmian et al. 2016, PN-90/A-75101/04]. To 2.5 g of the crushed sample 25 ml of distilled water was added and slowly heated on a hot plate. The solution was filtered and made up to 50 ml and was titrated with a 0.1M NaOH solution to obtain a pH 8.1. The results are presented as g citric acid (C₆H₈O₇) per 1 g of dry sample substance.

Statistical analysis

The obtained results were statistically analysed with Statistica 13.1 software. In order to determine the biochemical changes in kiwiberry fruits during storage were used two-factors ANOVA. The Tuckey HSD test was applied to identify statistically significant differences between the mean values at a confidence level of 95% ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The acidity of the fruit tested one day after rupture differed significantly (Fig. 1). The acidity of the fruit tested one day after rupture differed significantly. On the basis of the results, it was observed statistically important increase of TA after 5 days of storage in samples stored at 15 and 25°C, and after next 5 days amount of TA in the lowest and highest temperature is still at the same level. Krupa et al. [2011] studied the ripe *Actinidia arguta* fruit stored in refrigerated conditions for 42 days. Claimed the loss of acidity of these fruits with the experiment parameters used. Idaszewska et al. [2019] observed that the natural course of the life processes of blueberries resulted in a decrease in the content of citric acid from 1.1 to 0.7% and a significant increase in the content of ascorbic acid from 11.0 to 11.5 mg% for blueberries stored for 7 days. The authors found that overall of the organic acids decreases during the maturation of fruits [Idaszewska et al. 2019]. According to Kaliś [2015], the degradation of ascorbic acid depends on the content of enzymes in plant tissue. Moreover, high temperatures and long storage times can accelerate its degradation. Idaszewska et al. [2014] examined the impact of storage conditions (different temperatures and access or lack of light access) on the maturation of avid tomatoes. Drealized that the citric acid content in the initial stages of storage of tomatoes increased



Mean values for each storage time followed by the same letter do not differ significantly at $\alpha = 0.05$ (Tukey HSD test) – Wartości średnie dla danego czasu przechowywania, oznaczone tymi samymi literami nie różnią się istotnie przy $\alpha = 0,05$ (test Tukeya).

Fig. 1. Changes of total acidity of minikiwi fruits during storage at different temperature

Rys. 1. Zmiany kwasowości ogólnej w owocach minikiwi podczas przechowywania w różnej temperaturze

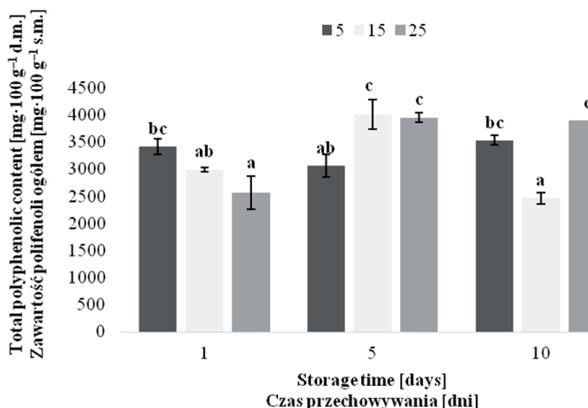
slightly. However, with the progressive process of maturation, the content of simple sugars increased, which down the concentration of citric acid.

The content of polyphenolic compounds (Fig. 2) in the fruit of the minikiwi varied according to storage conditions and ranged from 2,456 to 4,011 mg GAE·100 g d.m.⁻¹. On the first day of the study after harvest their content was higher at lower temperature. After 5 days, an increase in the case of samples stored at 15 and 25°C and a decrease in the content could be observed after another 5 days of storage of samples stored at 15°C. After 10 days the polyphenols content in samples stored at 5 and 25°C did not differ significantly. Such differences may be due to the lack of homogeneity of minikiwi fruit samples tested.

According to Krupa et al. [2011], the initial increase in the overall polyphenol content of Actinidia fruit, stored at 1°C for 42 days, was caused by a change in temperature and a thermal shock resulting from this. After the further weeks of storage, however, they observed a decrease in the content of polyphenol compounds. Latocha et al. [2015] noted that the skin of the fruit of the minikiwi is a rich source of phenolic compounds. Thus, low-total fruits, characterized by a significant share of the peel, showed higher content of these compounds.

The flavonoid content of the samples tested varied widely (Fig. 3). Significantly higher flavonoid content was characterized by samples stored at higher temperatures compared to those stored refrigerated. In addition, it can be seen that on the last day of the study the flavonoid content was lower than after half the time, where the highest flavonoid content (526 and 579 mg of quercetin per 1 g dry matter) was reported.

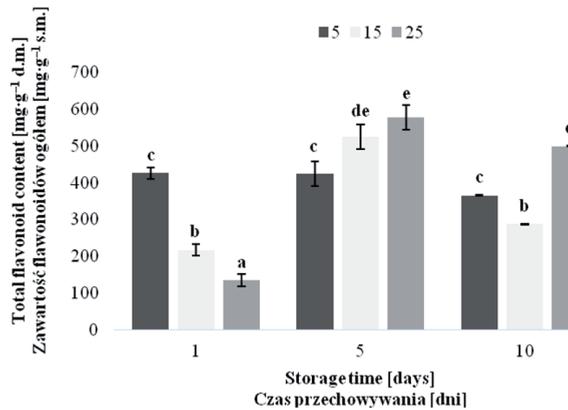
Stefaniak et al. [2017] reported that the total phenolic content of minikiwi fruits increased during storage under refrigeration conditions, in particular after four weeks. Valero et al. [2011] observed that in the cherries (sweet cherry) treated after harvesting



Mean values for each storage time followed by the same letter do not differ significantly at $\alpha = 0.05$ (Tukey HSD test) – Wartości średnie dla danego czasu przechowywania, oznaczone tymi samymi literami nie różnią się istotnie przy $\alpha = 0,05$ (test Tukeya).

Fig. 2. Changes of total polyphenolic content of minikiwi fruits during storage at different temperature

Rys. 2. Zmiany zawartości polifenoli ogółem w owocach minikiwi podczas przechowywania w różnej temperaturze



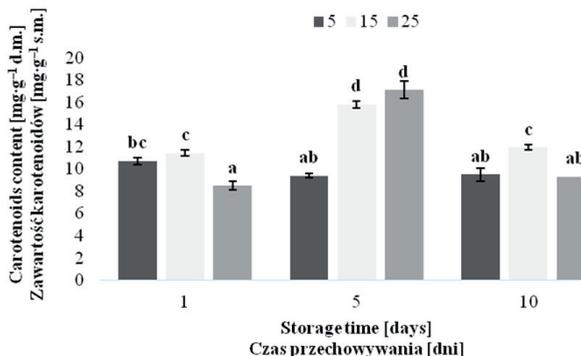
Mean values for each storage time followed by the same letter do not differ significantly at $\alpha = 0.05$ (Tukey HSD test) – Wartości średnie dla danego czasu przechowywania, oznaczone tymi samymi literami nie różnią się istotnie przy $\alpha = 0,05$ (test Tukeya).

Fig. 3. Changes of total flavonoid content of minikiwi fruits during storage at different temperature

Rys. 3. Zmiany zawartości flawonoidów ogółem w owocach minikiwi podczas przechowywania w różnej temperaturze

different acids, the increase in the concentration of phenolic compounds during storage is due to the post-harvest maturation process. Rapisarda et al. [2008] also noted an increase in flavonoid content in other refrigerated fruit (five orange genotypes), while slightly reducing the content of ascorbic acid.

Changes in the content of carotenoids during storage under different conditions are shown in Figure 4. Significant effects of time were found, as well as storage temperatures



Mean values for each storage time followed by the same letter do not differ significantly at $\alpha = 0.05$ (Tukey HSD test) – Wartości średnie dla danego czasu przechowywania, oznaczone tymi samymi literami nie różnią się istotnie przy $\alpha = 0,05$ (test Tukeya).

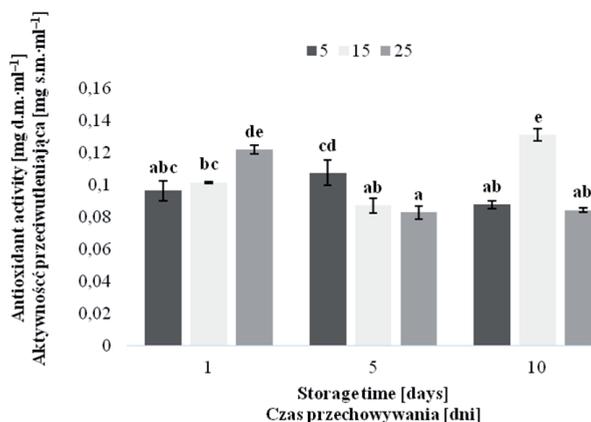
Fig. 4. Changes of carotenoid content of minikiwi fruits during storage at different temperature

Rys. 4. Zmiany zawartości karotenoidów w owocach minikiwi podczas przechowywania w różnej temperaturze

on the content of carotenoids. During the first fruit storage period, the content of the test samples increased, in particular when higher ambient temperatures are used. After another 5 days, a value of the compounds tested decreased. The shorter storage time of the fruit allowed to maintain a higher carotenoids content, which are beneficial for nutritional reasons.

Tavarini et al. [2008] examined the content of bioactive compounds in *Actinidia deliciosa* ‘Hayward’ during two storage variants (2 or 6 months of refrigerated storage with the last week under room temperature conditions). The content of carotenoids in the fruit was found to have increased in the first storage variant, from the time when the carotenoid content decreased in the second.

The storage time and temperature had a significant impact on the antioxidant capacity of the harvested fruit (Fig. 5). A marked increase in antioxidant capacity occurred during storage at higher temperatures. In the first 5 days, the antioxidant capacity significantly increased, which illustrated a decrease in the value of ABTS. In the next stage of storage these properties did not change for the sample stored at the lowest temperature. For a sample at 15°C after 10 days of storage the EC50 value was significantly higher than at 1 day. Increased antiradical activity results not only from the fruit ripening process, during which the content of biologically active compounds increases. The above theory is also confirmed by Mannozi et al. [2018]. Researchers in their studies stored blueberries for 2 weeks at 4°C and noticed an increase in antioxidant activity of fruit. Latocha et al. [2015] found that the total antioxidant activity of the transient fruit of the kiwi fruit depends on the species and variety. Tavarini et al. [2008] in their research proved that the antioxidant capacity of *Actinidia deliciosa* ‘Hayward’ fruit stored in refrigeration conditions has been reduced and its re-growth is possible



Mean values for each storage time followed by the same letter do not differ significantly at $\alpha = 0.05$ (Tukey HSD test) – Wartości średnie dla danego czasu przechowywania, oznaczone tymi samymi literami nie różnią się istotnie przy $\alpha = 0,05$ (test Tukeya)

Fig. 5. Changes of antioxidant activity (EC50) of minikiwi fruits during storage at different temperature

Rys. 5. Zmiany aktywności przeciwutleniającej (EC50) w owocach minikiwi podczas przechowywania w różnej temperaturze

by storing for a week under room temperature conditions. In addition, Mannozi et al. [2018] stressed that the ABTS method is a useful tool for determining the antiradical activity of berries.

CONCLUSIONS

Studies conducted indicate that the biochemical properties of kiwiberries depend on storage conditions. The value of bioactive compounds may increase or decrease over storage time. In the initial stage of storage of fruit, their content increased, while in the next period a decrease in the content of the compounds tested was observed. This was followed by the fluctuating antioxidant activity, which reached the highest values on the last day of the study.

In addition, the use of higher temperatures significantly accelerates the ripening of the fruit, which contributes to an increase in, and in the case of higher temperatures, to a constant content of total polyphenolic compounds. Which biochemical changes may also occur as a result of transpiration during storage, which depends mainly on the species of fruit tested, the degree of maturity and the conditions for their storage.

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ZMIANY WŁAŚCIWOŚCI BIOCHEMICZNYCH MINIKIWI PODCZAS PRZECHOWYWANIA

Streszczenie. Celem pracy było określenie wpływu czasu oraz temperatury przechowywania na właściwości biochemiczne owoców aktinidii ostrolistnej 'Weiki'. Owoce aktinidii przechowywano w temperaturze 5, 15 i 25°C przez 10 dni od momentu pozyskania surowca. W materiale oznaczano kwasowość miareczkową, zawartość flawonoidów oraz karotenoidów, całkowitą zawartość związków polifenolowych oraz aktywność przeciwutleniającą. Stwierdzono, że zarówno temperatura, jaki i czas przechowywania owoców miały statystycznie istotny wpływ na właściwości biochemiczne owoców minikiwi. Zastosowanie wyższej temperatury powodowało przyspieszenie procesu dojrzewania, co spowodowało obniżenie kwasowości owoców. Dodatkowo podczas przechowywania w wyższej temperaturze odnotowano wzrost zawartości cennych związków, w tym flawonoidów mających korzystny wpływ na zdrowie człowieka.

Słowa kluczowe: przechowywanie, *Actinidia arguta*, pojemność przeciwutleniająca, zawartość polifenoli ogółem, flawonoidy, karotenoidy