EFFECT ON PHYTOCHEMICAL CONTENT AND MICROBIAL CONTAMINATION OF ACTINIDIA FRUIT AFTER SHOCK COOLING AND STORAGE

– Research paper –

Monika FIGIEL-KROCZYŃSKA, Ireneusz OCHMIAN¹

Department of Horticulture, West Pomeranian University of Technology Szczecin, Słowackiego 17 Street, 71-434 Szczecin, Poland;

Monika Figiel-Kroczyńska; monika.figiel-kroczyńska@zut.edu.pl ORCID 0000-0003-4378-4004; Ireneusz Ochmian; iochmian@zut.edu.pl, ORCID 0000-0002-3606-1927,

ABSTRACT: Mini kiwi fruits are tasty and contain valuable nutrients - vitamin, micro and macroelements and polyphenols. The tested cultivars (Sientaibrska, Geneva, Issai, Ken's Red) belong to two species of Actinidia (*A. arguta* and *A. kolomikta*), which tolerate well the conditions of a temperate climate with negative temperatures in winter. The effect of postharvest shock cooling on fruit quality was investigated after 6 weeks of storage in CA and then after 5 days of shelf life. Shock cooling of fruit after harvest reduced adverse changes in fruit quality after storage in CA cold storage and shelf life. They were firmer, more puncture resistant, and retained more L-ascorbic acid and polyphenols. After 6 weeks of cold storage in CA, eight types of fungi and molds were found that caused the mycotoxins patulin, deoxynivalenol, and zearalenone were found in the fruit of all cultivars. Fruits of the Ken's Red cultivar were the most firm and puncture resistant, the darkest, and contained the most anthocyanins and the least L-ascorbic acid. In contrast, fruits of the cultivar Sientaibrska were the least suitable for storage and transport.

Key words: *Actinidia arguta*, *Actinidia kolomikta*, colour, firmness, polyphenols, CA storage, shelf life

INTRODUCTION

The genus *Actinidia*, popularly known as kiwi, is native to China and has about 70 species (Zhang, 2020). The name refers to the New Zealand bird of the same name. The most common species in this genus are *Actinidia deliciosa*, *A. chinensis*, *A. eriantha*, *A. arguta*, *A. kolomikta* and *A. purpurea*, (Chesonien et al., 2003). *A. arguta* and *A. kolomikta* are perennial, mostly dioecious plants that can also successfully grow in cooler regions because of their excellent freeze tolerance (Latocha, 2017). These species are native to Asia and are mainly cultivated there (Guan et al., 2011). Commercially, the fruits of these species are sold as: kiwi, hardy kiwi, baby kiwi, grape kiwi or mini kiwi (Nishiyama et al., 2004a) (Latocha, 2017). Mini kiwi fruits are small, smooth, and hairless, sweeter and more flavourful than the world's most common kiwi (*A. chinensis*). This makes them easy to peel and consume whole (Nishiyama et al., 2004a) (Latocha, 2017). They can be eaten raw whole, as

jam, or made into wine. The fruit is mostly green, but some cultivars with red skin colouring are also available (Latocha, 2017).

Due to its high nutrient and vitamin content, mini kiwi ranks among the top fruits classified as superfoods (Latocha, 2017). Mini kiwi fruits show high antioxidant and anti-inflammatory activity (Drummond, 2013) (Baranowska-Wójcik and Sz wajgier, 2019). In China, the leaves and fruits of *A. kolomikta* are used as a treatment for diabetes (Guan et al., 2011) (Lu et al., 2019). Studies by Zuo et al., 2012, show that *Actinidia* fruits may be a potential source of natural antioxidants. The antioxidant activity of fruits is associated with three main groups: vitamins, phenols, and carotenoids (Thaipong et al., 2006). Antioxidants sourced from fruits and vegetables are safer and healthier than synthetic ones (Zuo et al., 2012). *Actinidia* fruits are a valuable source of polyphenols, which are natural antioxidants. The fruits have industrial purposes (Wang, 2013) (Baranowska-Wójcik and Sz wajgier, 2019). Polyphenols are potent antioxidants and may exhibit chemo-resistance,

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¹ Corresponding authors. E-Mail address: iochmian@zut.edu.pl

anti-carcinogenic and anti-inflammatory properties, protect against certain cancers and reduce the risk of diabetes (Jing et al. 2008) (Kamiloglu et al. 2015). Total phenolic content in *A. kolomikta* and *A. arguta* fruits is up to 430 mg 100 g⁻¹ FW (Baranowska-Wójcik and Sz wajgier, 2019). One of the main antioxidants in fruit is *L*-ascorbic acid (vitamin C) (Nishiyama et al., 2004a). Free radicals cause many diseases, including cancer, heart disease, vascular disease, and neurodegenerative diseases (Fiorentino et al., 2009) (Muriel, 2009). Vitamin C is one of the major antioxidants in human body fluids (Kim et al., 2018) (Baranowska-Wójcik and Sz wajgier, 2019). The lack of the enzyme gulonolactone oxidase results in humans and great apes being unable to synthesise ascorbic acid (Naidu, 2003). As a result, this vitamin must be supplemented mainly through fruits and vegetables. The vitamin C content of the fruit can be up to 430 mg/100 g FW (*A. arguta*), making it one of the richest sources of vitamin C (Latocha, 2017).

Mini kiwi fruits also contain the highest level of B8 (myo-inositol) - up to 0.982 g/100 g FWB and other vitamins of B group among foods. These fruits are the richest source of lutein as well (Nishiyama et al., 2005). Lack of lutein and zeaxanthin can cause blindness associated with cataracts and age-related macular degeneration (AMD) (Abdel-Aal et al., 2013). Fruits are a source of vitamin E (4.6-5.3 mg/100 g FW) and β -carotene (Nishiyama et al., 2008) (Latocha, 2017) (Zuo et al., 2012). Due to their high dietary fibre content, consumption of *A. arguta* and *A. kolomikta* fruits normalises intestinal peristalsis and promotes metabolism (Nishiyama et al., 2004b). From among many fruits including the commonly known kiwi fruits (*A. deliciosa* or *A. chinensis*), *A. arguta* cultivars are the greatest source of promoting compounds (Latocha, 2017). In recent years, there has been a significant increase in the market demand for high-quality fruit. Therefore,

it is important to assess fruit ripeness and storage shelf life (Gomez et al., 2008). The shelf life of fruit after harvest is determined by appearance (freshness, colour, absence of rot or physiological disorders) and texture (firmness, juiciness, and crispness) (Ayala-Zavala et al., 2004). The method of storage of fruit is very important for its quality and especially for its organoleptic taste characteristics (Barboni et al., 2010). During fruit storage, quantitative losses (weight loss) and qualitative changes occur, which affect the consumer and nutritional value, e.g. decrease in vitamin content, texture, colour, or flavour (Krzysztofik and Łapczyńska-Kordon, 2008). *A. arguta* fruits have a short shelf life (Latocha et al., 2014) and can be stored under refrigeration (a storage life under refrigeration 0 °C, 90-95% RH) for only 2 months. Initial visual assessment of fruit can influence consumer decisions. Characteristics such as colour and firmness mainly determine purchase and consumption. Fruit firmness is a cultivar-specific trait, but it also depends on fruit size (Ochmian and Kozos, 2015). This trait plays a key role during storage and transport. It affects the resistance to mechanical damage. During fruit ripening, its susceptibility to pathogens increases (Valero et al., 2002). Fungal diseases are responsible for the loss of food products (vegetables and fruits) during storage. This is a problem for producers, retailers and consumers. Because due to the mycotoxin presence that poses a danger to human and animal health (Prakash et al., 2015) (Sanzani et al., 2016).

The aim of this experiment is to determine the effect of shock cooling on physico-chemical changes (firmness, color, *L*-ascorbic acid, polyphenols) in mini kiwi fruit after storage period. The type and amount of microbiological contamination and mycotoxin in the fruits were also analyzed.

MATERIAL AND METHODS

Characteristics of the area of research and plant material

The experiment was conducted in the Department of Horticulture at West Pomeranian University of Technology in Szczecin. Four cultivars belonging to two species of the Actinidia family were studied in the experiment: *Actinidia arguta* cultivar Sientiabrskaja and *Actinidia kolomikta* cultivars Geneva, Issai, and Ken's Red.

Fruit storage in cold storage CA

Fruits of the tested cultivars, selected for uniform size and absence of visible defects, were harvested at of 9-10 °Brix. This is the recommended SSC level for fruit intended for long storage (Latocha et al., 2014).

Both the shock-cooled mini kiwi fruit (temperature drop to 3-4 °C within 2 hours after picking) and not shock-cooled mini kiwi fruit were then stored in a cold room with a controlled atmosphere (1.5; 1.5). Berries were stored for 6 weeks at a temperature of 1.5±0.25 °C and relative air humidity of 95%. The experiment was performed in five repetitions, each for 1.25 kg of berries.

After six weeks of cold storage in a controlled atmosphere, the fruit was transferred to a room with simulated conditions (shelf life), where it was stored for another five days.

The physical features of fruits (puncture of the skin and firmness), colour, antioxidant activity, and *L*-ascorbic acid were measured on fresh berries. Phenolic samples then were kept frozen (-27 °C) in polyethylene bags (3 × 250 g) until analysed.

Colour

CIE *L*a*b** (Color Measurement Committee of the Society of Dyers and Colorists) was measured using a spectrophotometer KonicaMinolta CM-700d. The measured parameters were *L** (lightness/darkness), *a** (red/green) and *b** (yellow/blue). The colour parameters and indices were averaged over 35 measurements.

Firmness

Firmness and puncture resistance of the berry skin was measured with a FirmTech2 apparatus (BioWorks, USA) on 100 randomly selected berries from three replicates and was expressed as a gram-force causing the berry surface to bend 1 mm. Punctures were made using a stamp with a diameter of 3 mm.

Extraction procedure and identification of phenolic compounds, antioxidant activity

Fruits were then prepared and analyzed according to the methodology of Oszmiański et al. (2018). The fruits were extracted with methanol acidified with 2.0% formic acid. Separation was conducted twice by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland). The sample was shaken several times. Subsequently, the suspension was centrifuged (MPW-251, MPW MED. INSTRUMENTS, Warsaw, Poland) at 19 000×g for 10 min. Prior to analysis, the supernatant was additionally purified with a Hydrophilic PTFE 0.20 µm membrane (Millex Simplicity Filter, Merck). All extractions were carried out in triplicate.

Analyses were performed according to the methodology of Oszmiański et al. (2018). In kiwiberry fruit extracts, polyphenol identification was executed using an ACQUITY Ultra Performance LC system appointed with a binary solvent manager, a photodiode array detector (Waters Corporation, Milford, MA, USA) and a G2 Q-TOF micro mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in both negative and positive modes.

Antioxidant activity

The ferric reducing antioxidant property (FRAP) was also determined (Benzie and Devaki, 2018). The antioxidant capacity was expressed as millimoles of Trolox per 100 g distilled water. FRAP assay measurements were carried out on a UV-2401 PC spectrophotometer. *L*-ascorbic acid content was measured with a RQflex 10 requantometer (Merck).

Fruit infestation by moulds and yeasts

Fruits were collected at the time of harvesting. The research material was placed in sterile plastic containers. The material was subjected to microbiological analysis immediately after harvesting and after cold storage. The analysis of the degree of fruit infestation by fungi (yeasts and moulds) was based on the European standard ISO (Anonymous, 2008). After the cultivation of spore-forming fungal inoculates, they were subjected to taxonomic evaluation using the traditional method of macroscopic observation of colonies and microscopic observation of spores and filaments (Ulbin-Figlewicz et al., 2015). The fungi were identified to the genus level. Colonies were counted using an automatic colony counter (Alchem PCC04). Analyses were performed on three replicates from each sample.

The content of mycotoxins in fruits

Mycotoxins were determined using HPLC with MS/MS detection (Błajet-Kosicka et al., 2014). The sample was purified on the AflaTest immunological affinity columns from Vicam for aflatoxins and OchraPrep from R-Biopharm Rhône Ltd for ochratoxin A, according to the procedure specified by the manufacturer. Patulin, deoxynivalenol, T2, HT2 toxin and zearalenone were analyzed by HPLC-MS/MS. The samples were purified on Bond Elut® Mycotoxin columns from Varian. Each sample was subjected to 3 repetitions.

Statistical Analysis

All statistical analyses were performed using Statistica 13.1 (StatSoft Polska, Cracow, Poland). Nonparametric methods (Kruskal–Wallis test) were used if neither the homogeneity of variance nor the normality of distribution was established previously. Statistical significance of the differences between means was determined by testing the homogeneity of variance and normality of distribution, followed by ANOVA with Tukey's post hoc test. The significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Firmness and puncture

The firmness of currently produced kiwi fruits allows them to be stored and transported (White et al., 2005). During long-term storage of *A. argute* and its hybrid fruits, the strongest changes in fruit characteristics were related to fruit firmness (Latocha et al., 2014) (Figiel-Kroczyńska et al., 2021). Fruit firmness determines their resistance to mechanical damage (Ochmian and Kozos 2015). Mini kiwi fruits lose firmness during storage and their puncture decreases (Table 1). This may be related to changes in the cell wall and water loss (Jiang, et al., 2016) (Kim et al., 2018). Post-harvest water loss and turgor pressure drop are irreversible, due to the inability of the cell to re-hydrate. The *Actinidia* cultivars studied were characterised by high variability in initial as well as post-storage firmness. Fresh fruit of Ken's Red cultivar was the most firm, 35% firmer than the fruit of the Sientiabrskaja cultivar. White et al. (2005) also noted that the small-fruited genotypes of *Actinidia* are highly variable in terms of final firmness. By applying shock cooling technology to the fruits after harvesting, we prolong their shelf life. Reducing respiration has a positive effect on inhibiting fruit ripening, i.e. starch hydrolysis, chlorophyll degradation, and fruit softening (Lim et al., 2016). Without shock-cooling after shelf life, fruit firmness decreased on average by 23%, while using shock-cooled technology fruit firmness decreases by 13.3% compared to fresh fruit. Latocha et al. (2014)

found that fruit firmness decreased by about 25-30% after 4 weeks of storage in CA. The rapid loss of firmness becomes apparent when the fruit is removed from cold storage and kept at higher temperatures, making it difficult to transport and sell the fruit (Salvador et al., 2004).

Polyphenolic compounds

Four subclasses of polyphenols - anthocyanins, phenolic acids, flavonols, and flavan-3-ols - were found among the four *Actinidia* cultivars studied (Figure 1). The cultivar richest in polyphenols was Sientiabrskaja, followed by Ken's Red, Issai, and Geneva. All four studied cultivars had the most flavan-3-ols. The next rank in terms of content in fruit of the cultivars Sientiabrskaja, Geneva and Issai were phenolic acids > flavonols > anthocyanins. Especially 'Sientiabrskaja' was rich in flavan-3-ols, which made up 57.3% of all polyphenols in fresh fruit. In the study by Wojdyło et al. (2017), also flavan-3-ols were the major class of polyphenols in *Actinidia* species but accounted for as much as 96-99% of all polyphenolic compounds. Next were flavonols (1-4%) > phenolic acids (0.4-0.7%) and anthocyanins (0-0.8%). 'Ken's Red' fruit also contained a lot of anthocyanins (14.6 mg/100 g, i.e. 31.8% of all polyphenols). A significant amount of these compounds was also found in fruits of the Geneva cultivar - 3.2 mg/100 g. The content of this group of polyphenols was reflected in the colour of fruits of these two cultivars - they were maroon-red or had red blush.

Table 1. Changes in fruit firmness after storage in a CA for 6 weeks and simulated 5-day shelf life

		Sientiabrskaja	Geneva	Issai	Ken's Red	Mean
After storage and shelf life (5 days)	Firmness (G/mm)					
	fresh fruit - control	126c*	178c	162d	194d	165C
	After storage	Not shock-cooled	115bc	167c	149bc	152B
		shock-cooled	121c	173c	153cd	158BC
		not				
	After storage and shelf life (5 days)	shock-cooled	87a	140a	133a	127A
		shock-cooled	104b	155b	145ab	143B
	Puncture (G/mm)					
	fresh fruit - control	79b	143d	121d	135d	120C
After storage and shelf life (5 days)	After storage	Not shock-cooled	75b	129bc	106c	109BC
		shock-cooled	77b	135cd	115cd	114C
	After storage and shelf life (5 days)	Not shock-cooled	55a	111a	77a	87A
		shock-cooled	68ab	124b	93b	101B

*Values followed by different letters in columns are significantly different at 5% level based on Tukey's test

Figure 1. Polyphenols derivative contents in Actinidia fruits after storage in CA for 6 weeks and simulated 5-day shelf life

Anthocyanins, which are mostly located in berries skin, are the reason of colouring them to the red, blue, or purple. They also characterised by high antioxidant activity (Ribera et al., 2010) (Wojdyło et al., 2017). After six weeks of storage, the anthocyanin content increased slightly in these cultivars. This may have been caused by the increased rate of fruit ripening, as evidenced by changes in colour parameters (mainly a^*) and a decrease in fruit firmness. The fruits were more red than fresh fruits.

After 6 weeks of storage, the content of polyphenols in the fruit of all cultivars studied changed. Generally, there was a decrease in the content of these compounds. The storage of fruits in a cold store influences a significant reduction of polyphenols (Ochmian et al., 2020). Only in 'Kens Red' fruits that were not pre-cooled, an increase in polyphenols was observed in all groups, especially in anthocyanins. The use of shock cooling technology for post-harvest fruit reduced polyphenol losses during storage. The highest losses of polyphenols occurred during simulated shelf-life storage, but shock cooling allowed to reduce these losses. Shock cooling of fruit after harvest is a technological process that preserves fruit quality (Kozos et al., 2014).

***L*-ascorbic acid and antioxidant activity**

Mini kiwi is a fruit that has a great influence on human body, as it is source of many natural bioactive components, which has health-promoting effect.

Among them, there are non-enzymatic antioxidants: vitamins and polyphenols. They are the main compounds affecting the antioxidant activity of these fruits (Valko et al., 2006) (Krupa et al., 2011) (Leontowicz et al., 2016).

Actinidia fruits, especially hybrids such as Issai are the rich source of vitamin C, what is proved by a lot of scientific publications (Nishiyama et al., 2004a) (Drummond, 2013) (Leontowicz et al., 2016) (Latocha, 2017). In the present study, it was found that the *L*-ascorbic acid content was highest in fresh fruit and averaged 60.5 mg/100 g for the cultivars studied (Table 2). This was within the range obtained by Nishiyama et al. (2004a), (37.3 - 184.6 mg/100 g FW). The content decreased on average by as much as 20.1% after shelf life, for non-shock-cooled fruit. However, shock cooling of fruit after harvest resulted in a slower decrease in *L*-ascorbic acid content by 4.2% after storage under CA conditions and another 7% after shelf life simulated conditions. The results obtained are consistent with those obtained by Tavarini et al. (2008), who also found a decrease in vitamin C in fruit after storage.

Table 2. Changes in *L*-ascorbic acid content and antioxidant activity of fruit after storage in CA for 6 weeks and simulated 5-day shelf life

			Sientiabrskaja	Geneva	Issai	Ken's Red	Mean
<i>L</i> -ascorbic acid (mg/100) fresh fruit - control			72.3bc	55.7d	61.2c	52.7c	60.5C
After storage and shelf life (5 days)	After storage	Not shock-cooled	69.0ab	52.3cd	54.3b	45.1b	55.2B
		shock-cooled	75.8c	50.5bc	60.0bc	43.9b	57.6B
		Not shock-cooled	63.6a	44.2a	47.1a	36.3a	47.8A
		shock-cooled	68.9a	47.9ab	55.6b	42.4b	53.7B
FRAP (μmol/T g) fresh fruit - control			13.6a	19.2a	25.0a	15.8a	18.4A
After storage and shelf life (5 days)	After storage	Not shock-cooled	17.3b	25.8b	28.3a	17.9a	22.3B
		shock-cooled	14.8a	20.7a	28.8ab	15.3a	19.9AB
		Not shock-cooled	20.1b	30.1c	36.0c	22.8b	27.3C
		shock-cooled	18.9b	19.7a	30.4b	18.1a	21.8B

*Values followed by different letters in columns are significantly different at 5% level based on Tukey's test

The FRAP method was used to determine the antioxidant capacity of Actinidia fruit. A high antioxidant capacity of mini kiwi fruits was found. Among the tested cultivars, Sientiabrskaja had the highest antioxidant potential, while Issai had the lowest. Shock-cooled fruit had a significantly higher antioxidant activity, compared to fruit not subjected to this process. After 6 weeks in CA, antioxidant activity decreased by 21%. The highest dynamics of changes were observed after shelf life - after 5 days the fruits lost another 27% of their free radical binding capacity. A relationship was observed between the method of fruit storage and the decrease in vitamin C content and FRAP antioxidant capacity. The antioxidant potential measured by the FRAP method depended more on the vitamin C content than on the phenolic compounds (Wojdyło et al., 2017).

Fruit color

One of the main parameters affecting consumer acceptance of the fruit is its colour. The colour of kiwi fruit skin and flesh is variable between cultivars (Latocha, 2017). The majority of mini kiwi fruits found to be green, however, some cultivars

with red blush and fruits with brown-red color are also present. The color of the studied fruits was determined by the parameter L^* , a^* and b^* (Ochmian et al., 2012). The lightest and greenest fruits were of the Sientiabrskaja cultivar, while the darkest and most reddish-brown fruits were of Ken's Red cultivar (Figiel-Kroczyńska et. al., 2021). This is related to the high content of anthocyanins. Storage of fruit caused fruit darkening, as indicated by the L^* parameter. This indicates a progressive ripening of the fruit. Shock cooling inhibits life processes in fruit, which affects the slower change of fruit colour during storage (Fig. 2). There was a change in the colour of the fruit skin during storage. All the fruits were darker in comparison with fresh fruits. 'Sientiabrskaja's' fruits turned dark green. The fruit colour of 'Issai' changed towards yellow and 'Ken's Red' towards dark red. Changes in the value of the a^* parameter were most visible in the cultivars Geneva and Ken's Red. The changes that occurred in the colour are a natural phenomenon that takes place during fruit storage. Fruits lose their green colour during storage and this process occurred more slowly when they are properly stored e.g. in a CA cold store (Szpadzik et al., 2021).

n – not shock-cooled. s - shock-cooled. f - fresh fruit – control. s-l - shelf life
S – Sentiabrskaja. G – Geneva. I – Issai. K - Ken's Red

Figure 2. Change in fruit colour after CA storage for 6 weeks and simulated 5-day shelf life

Mould and yeast microflora

The number of yeasts isolated from fruit varied widely (1.6-4.3 log₁₀ CFU/g) (Table 3). In the cultivars, Sentiabrskaja and Ken's Red the number of yeasts isolated from fruit stored in CA for 6 weeks was lower than from fresh fruit. Storage of fruits for 5 days in shelf life did not affect the number of yeasts to a significant extent. A significant increase was observed only on 'Sentiabrskaja' fruit. On Sentiabrskaja fruit, both fresh and after storage, the highest number of yeast

was found (3.5-4.3 log₁₀CFU/g). The least yeast, but also mould was on Ken's Red fruit - 1.6-2.1 and 1.7-2.0 log₁₀CFU/g, respectively.

Saprophytic genera (*Penicillium*, *Alternaria*, *Acremonium*, *Mucor*, *Aureobasidium*) and phytopathogenic fungi (*Bipolaris* sp., *Botrytis cinera*, *Phomopsis vaccinii*, *Phytophthora* sp., *Rhizoctonia solani*, *Cladosporium* sp., *Fusarium* sp.) are most commonly found on fruit (Bensch et al., 2015). These fungi are commonly found on fruit and in soil (Tournas and Katsoudas, 2005)

(Kućmierz et al., 2013). Fresh fruits, including Actinidia, are prone to fungal infections, which occur either on the plantation or in cold storage or transport. The fruits contain a lot of simple sugars and organic acids, which is a good breeding ground for yeasts and moulds. Infections adversely affect the visual appearance of the fruit and its composition, as well as its nutritional value. Fungi belonging to eight different genera (*Cladosporium*, *Fusarium*, *Penicillium*,

Acremonium, *Alternaria*, *Aureobasidium*, *Bipolaris* and *Mucor*) were isolated from fresh fruit and only five fungal genera from stored fruit. Although more fungal genera were found on fresh fruit, the vast majority belonged to one dominant genus, i.e. *Cladosporium* (Table 3). On the fruit of all minikiwi cultivars, fresh and stored, they amounted to 38-93% of all identified fungi. After storage, fungi of the genera *Aureobasidium* and *Fusarium* were found to increase in abundance (Table 4).

Table 3. Impact of the storage of Actinidia fruit on the amount of yeast and mould

		Sientiabrskaja	Geneva	Issai	Ken's Red	Mean
Fresh fruit - control	Yeast log ₁₀ CFU/g	4.0c/B^a	3.9c/A	2.7b/A	2.1a/B	3.2A
	Mould log ₁₀ CFU/g	3.3b/B	3.5b/A	3.3b/A	1.9a/AB	3.0A
After storage	Yeast log ₁₀ CFU/g	3.5c/A	3.7c/A	2.9b/AB	1.7a/A	3.0A
	Mould log ₁₀ CFU/g	2.7b/A	3.5c/A	3.0b/A	1.7a/A	2.7A
After storage and shelf life (5 days)	Yeast log ₁₀ CFU/g	4.3d/B	3.9c/A	3.2b/B	1.6a/A	3.3A
	Mould log ₁₀ CFU/g	2.9b/AB	3.8c/B	3.2b/A	2.0a/B	3.0A

^aValues followed by the same letter, within the same column, were not significantly different ($p < 0.05$) according to the t-Tukey test; lower case letters in a line, capitals in column

Table 4. Impact of the storage of Actinidia fruit on the composition and variety of fungi as well as the presence of mycotoxins in fruit

		Sientiabrskaja		Geneva		Issai		Ken's Red	
Ffesh fruit - control	Percentage (%) of fungi genus	70	<i>Cladosporium</i>	67	<i>Cladosporium</i>	79	<i>Cladosporium</i>	89	<i>Cladosporium</i>
		18	<i>Fusarium</i>	15	<i>Aureobasidium</i>	10	<i>Fusarium</i>	6	<i>Alternaria</i>
		5	<i>Penicillium</i>	8	<i>Penicillium</i>	4	<i>Penicillium</i>	3	<i>Bipolaris</i>
		3	<i>Alternaria</i>	7	<i>Fusarium</i>	3	<i>Alternaria</i>	2	<i>Acremonium</i>
		2	<i>Acremonium</i>	2	<i>Alternaria</i>	2	<i>Acremonium</i>		
		1	<i>Bipolaris</i>	1	<i>Bipolaris</i>	1	<i>Bipolaris</i>		
		1	<i>Mucor</i>			1	<i>Eurotium</i>		
	Mycotoxins µg/kg	3.11	PAT	1.28	PAT	1.55	DON		
		1.13	DON	0.79	DON	0.73	PAT		
		0.13	ZEN ^b						
After storage	Percentage (%) of fungi genus	38	<i>Cladosporium</i>	52	<i>Cladosporium</i>	46	<i>Cladosporium</i>	93	<i>Cladosporium</i>
		28	<i>Aureobasidium</i>	44	<i>Fusarium</i>	38	<i>Fusarium</i>	7	<i>Penicillium</i>
		16	<i>Alternaria</i>	4	<i>Penicillium</i>	9	<i>Penicillium</i>		
		13	<i>Fusarium</i>			7	<i>Alternaria</i>		
		5	<i>Penicillium</i>						
	Mycotoxins µg/kg	0.55	PAT	0.64	PAT	0.22	DON	0.53	PAT
		0.09	ZEN ^b	0.23	DON				
After storage and shelf life (5 days)	Percentage of fungi genus	58	<i>Cladosporium</i>	81	<i>Cladosporium</i>	77	<i>Cladosporium</i>	79	<i>Cladosporium</i>
		19	<i>Aureobasidium</i>	10	<i>Penicillium</i>	9	<i>Fusarium</i>	14	<i>Penicillium</i>
		11	<i>Fusarium</i>	7	<i>Fusarium</i>	9	<i>Penicillium</i>	7	<i>Fusarium</i>
		9	<i>Penicillium</i>	2	<i>Alternaria</i>	3	<i>Alternaria</i>		
		3	<i>Alternaria</i>			2	<i>Aureobasidium</i>		
	Mycotoxins µg/kg	1.05	PAT	0.44	PAT	1.20	PAT	0.88	PAT
		0.17	ZEN ^b	0.43	DON	0.17	DON		

^aValues followed by the same letter, within the same column, were not significantly different ($p < 0.05$) according to t-Tukey test; ^bDeoxynivalenol – DON, Patulin – PAT, Zearalenone – ZEN

In samples where *Fusarium* fungi were isolated, Deoxynivalenol, a mycotoxin of the genus *Fusarium*, was also found (0.17-1.55 µg/kg in fresh and stored fruit) (Table 4). Zearalenone, which is a toxic secondary metabolite also from *Fusarium* fungi, was detected only in fresh Sientia-brskaja fruit (0.13 µg/kg) after storage (0.09-0.17 µg/kg). Patulin (0.44 - 3.11 µg/kg), a secondary metabolite of some mould species, including the genus *Penicillium*, was also detected in the fruit. Patulin is a toxin with mutagenic, teratogenic, and possibly carcinogenic properties. The World Health Organisation (WHO) and the European Commission have set a maximum concentration limit for patulin in apple juice of 50 µg/kg (EC, 2006). 6 weeks storage of fruit in CA at a temperature of 1.5 °C ± 0.25 °C, did not eliminate

Fusarium. This resulted in the presence in the fruit of Deoxynivalenol and Zearalenone. Chilling circumstances are not efficient in eliminating toxin-producing fungi (Alshannaq and Yu, 2017) (Tournas and Katsoudas, 2005). Ochratoxin A, T2 HT2 toxin, Aflatoxins (B1, B2, G1, G2) were not identified in fresh and stored fruit samples. The presence of mycotoxins in the tested fruits was found at a low level. This could be influenced by yeasts and fungi e.g. of the genus *Aureobasidium*. They can degrade mycotoxins through microbial pathways (Zhu, R. et al., 2015). They show strong antagonism towards moulds contaminating fruit and can provide effective bioprotection (Chi, Z. et al., 2009).

CONCLUSIONS

The physicochemical and health-promoting properties deteriorated during the storage of the mini kiwi studied cultivars *A. arguta* and *A. kolomikta*: firmness, puncture resistance, L-ascorbic acid level, polyphenol content, and antioxidant capacity (FRAP).

The results of the study show that shock cooling of mini kiwifruit improves their storage quality and extends shelf life.

Among the four cultivars studied, the fruit of Ken's Red was the most firm and puncture-resistant, the

darkest, and had the most anthocyanins, while L-ascorbic acid was the least. The fruits of the Sientia-brskaja cultivar were the least suitable for storage and transport.

To prolong the storage time and preserve the health-promoting properties of *A. arguta* and *A. kolomikta* fruit, the method of fruit shock cooling after harvesting should be used.

It is very important to test fruits for mycotoxins, especially after storage, before they reach consumers. After 6 weeks of cold storage in CA, the mycotoxins Patulin, Deoxynivalenol, or Zearalenone were found in the fruit of all cultivars.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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