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Content of biologically active substances in sweet cherry fruits at different stages of fruit development in the conditions of the living mulch

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Abstract. Content of soluble solids, sugars, titrated acids, ascorbate, glutathione, phenolic substances, anthocyanins, total reducing activity of fruits tissues in sweet cherry fruits studied at different stages of fruit development during 2018 and 2019 in an organic sweet cherry orchard (*Prunus avium L. / Prunus mahaleb*) in the Southern Steppe of Ukraine. The aim of the research was to determine how the living much conditions (compared to bare fallow) affect the content of biologically active substances in sweet cherry fruits at different stages of ripening. It was determined that the fruits of sweet cherry accumulated significantly more ascorbate, phenolic substances and anthocyanins in the conditions of living mulch (compared to the fruits of the trees on bare fallow). So, at the stage of picking ripeness, the content of ascorbate in sweet cherry fruits in the conditions of living mulch was 29 and 22% more compared to bare fallow (respectively, in 2018 and 2019), phenolic substances - by 47 and 23%, anthocyanins - by 36 and 26%. The revealed regularities can be explained by stressful conditions of competition with natural herbs, which activate the synthesis of anti-stress, antioxidant biologically active substances in plant tissues (including fruits). Since it is the antioxidants of the fruits that have a physiological value for humans, it can be stated that the fruits grown in the conditions of living mulch have a higher therapeutic and preventive value than the fruits grown on bare fallow.

Key words: sweet cherry; living mulch; sugars, titrated acids, ascorbate, glutathione, phenols.

INTRODUCTION

All over the world, sweet cherries are valued not only for their taste, but also for their antioxidant properties, which help people with the treatment of many diseases, including diabetes, obesity, Alzheimer's disease, hypertension, cancer (Gonçalves et al., 2019; Fonseca et al., 2021). Organic sweet cherry fruits contain significantly more sugars, anthocyanins, flavonoids, compared to conventional fruits (Hallmann & Rozpara, 2017). Mulch is recognized as the most soil-saving practice in horticulture, especially for organic orchards, as it protects the soil from water and wind erosion (Gomez et al., 2008; Fidalski et al., 2010; Atucha et al., 2013) creates optimal conditions for soil microbiota (Yao et al., 2005; Zheng et al., 2018; Culumber et al., 2019), lowers soil temperature in summer and insulates soil in winter (Gu et al., 2016; Gerasko et al., 2021), retains soil moisture (due to the increase of organic matter and, accordingly, increases the water holding capacity of the soil) (Palese, 2014; Simões et al., 2014; Zheng et al., 2018). Thus, applying living mulch as an orchard floor management system meets the goal of leaving fertile soil for future generations and a strategy for the development of sustainable agriculture (Holden et al., 2017). But the emergence of sustainable agriculture requires the efforts of not only the farmers but also the scientists through the creation of a scientific base, namely, the need to determine the impact of living mulch on the physiological processes of fruit trees in detail, in order to have a clear idea of the 'pros' and 'cons' for producers. Regarding the effect of living mulch on the physiological state of fruit trees, and on the biochemical composition of fruits, the data is contradictory: according to E.E. Sánchez et al. (2007), living mulch improves the quality of the fruits of *Malus domestica Borkh.* in northern Patagonia and increases biologically active substances content; A. Atucha, I.A. Merwin & M.G. Brown (2011) indicate a decrease in fruit quality under the influence of living mulch; the results of studies by G.H. Neilsen et al. (2014) indicate no effect of living mulch on fruit quality. The physiological state of plants depends on their ability to withstand various stresses (Anjum et al., 2010). Using cover crops in orchards can create additional stress for fruit trees due to the competition with grasses (Atucha et al., 2011). Long-term research indicates that over time, trees overcome competition with grasses (Merwin, 2010; Atucha et al., 2011), but how this process works, what changes occur in the physiological parameters of fruit trees, is not yet definitively clarified. Information on the content of biologically active substances in sweet cherry fruits under living mulch conditions will help to understand the physiological changes that occur in the tissues of fruit trees and how they manage to overcome competition with herbs.

The aim of this research was to determine how the living mulch conditions affect the content of sugars, titrated acids, ascorbate, glutathione, phenolic substances, anthocyanins in sweet cherry fruits at different stages of ripening.

MATERIALS AND METHODS

The field experiment was set up in the research orchard of the Tavria State Agrotechnological University (Zelene village, Melitopol district, Zaporizhzhya region: 46 °46 'N, 35 °17 'E). The soil of the experimental site is chestnut, sandy, with light mechanical composition. Soil solution has a slightly alkaline reaction (pH ranges from 7.1 to 7.4). The humus content in the upper layer is 0.6%, the total water-soluble salt content is 0.015–0.024%. Mineral nitrogen was not detected, the content of P_2O_5 is 5.4; K_2O - 6.5 mg kg^{-1} of the soil. Sweet cherry is a traditional fruit crop for this region and has excellent fruit quality on such poor sandy soils.

The research site is located in the Southern Steppe of Ukraine, which climate is characterized as arid and very warm. Weather conditions in the years of the research (2018, 2019) had higher mean annual air temperature than long-term data by 1.3–1.6 °C. April of 2018 was warm and very dry. The drought also lasted in May and June of 2018. July of 2018 was satisfactory in terms of precipitation, but August this year was very dry as well (the amount of precipitation was 82% lower than the long-term average). The heavy rainfall in September 2018 was followed by draught in October (the amount of precipitation was 44% less than the long-term average). April and May of 2019 were satisfactory in terms of moisture supply (the amount of precipitation was 44 and 107% higher than the long-term average, respectively). But June of 2019 was abnormally hot and dry. In general, it can be stated that the conditions of fruit ripening (from the stage of petals falling to the picking ripeness) in 2018 were abnormally arid, and in 2019 the period of fruit ripening was satisfactory in terms of moisture.

The research was carried out sweet cherry trees of 'Dilemma' cultivar grafted on *Prunus mahaleb* seedlings, planted in 2011 (7×5 m). 'Dilemma' cultivar is medium-early, in the conditions of Melitopol ripens in early June, is used for fresh consumption. The fruits are convex-heart-shaped, the skin and flesh are dark red, have excellent sour-sweet taste.

The experiment was designed as a randomized complete block with two variants, in three replications (10 control trees per replication). Orchard floor management system in the experimental plot had two variants: bare fallow (tilling to a depth of 15 cm, manual weeding) and living mulch (natural grasses, mowing, mown mass remained in place). The rest of the technological operations in the orchard were identical in both variants. Synthetic fertilizers and chemical plant protection products were not used.

Fruit samples collection for analysis was performed during their ripening (May-early June) in the following stages: petal fall, stone hardening, partial reddening, picking maturity. In order to determine the biochemical composition, 30 intact fruits were selected in four replications from each variant of the experiment.

The collected data included: the content of soluble solids (SS, %), sugars (Sug, %), titrated acids (TA, %), phenolic substances (Phen, mg GA 100 g^{-1}), anthocyanins (Ant, mg 100 g^{-1}), ascorbic acid (AsA, mg 100 g^{-1}), glutathione (Glu, mg 100 g^{-1}) and the total reducing activity of fruits tissues (TRA, mL KIO_3 100 g^{-1}) in sweet cherry fruits.

The content of soluble solids and titrated acids was determined in accordance with the Methods of determining the quality of crop products (Methods…, 2021) by desktop refractometer IRF-451 62M.

Determination of the number of sugars (%) in fruits tissues was performed photometrically based on the ability of monosaccharides to reduce picric acid (2,4,6-trinitrophenol) to picramic acid, where the reaction product has an intense red colour. The calibration graph was prepared by glucose. The optical density was determined at a wavelength of 490 nm (Workshop …, 2001).

The total content of phenolic substances was determined photometrically using Folin-Chokalteu reagent (Yaman, 2022a; Yaman, 2022b). The optical density of the mixture was measured at a wavelength of 765 nm, which corresponds to the concentration of phenolic substances in terms of gallic acid (GA). Total amount of phenolic compounds was expressed in mg of gallic acid per 100 g of fresh mass (mg GA 100 g^{-1}).

Analysis of the anthocyanin content was performed as described by Francis (1982): 1 g of crushed tissue was suspended in 10 mL of 1.5 N HCl solution in 85% ethanol, transferred to a 50 mL volumetric flask and extracted for 13 hours in the refrigerator in the dark. The extracts were filtered (Whatman filter paper #1) and the absorbance was measured at $\lambda = 535$ nm. Determination of the content of ascorbic acid, glutathione and the total reducing activity of fruits tissues was performed on the reducing properties of ascorbate and glutathione, as described by Gorodniy (2006): a portion of fresh mass (2 g) was thoroughly grounded in a mortar with 8–10 mL of 5% HPO_3 solution, quantitatively transferred to a 50 mL volumetric flask, bringing the contents to the mark with distilled water. After shaking for 2–3 minutes, filtered through a dry folded filter into a dry flask. 5 mL of the filtrate was titrated with 0.001 n Tilman's paint solution (2,6-dichlorophenolindophenol) to a faint pink colour that does not disappear for 60 s. Simultaneously, the other 5 mL of filtrate (after adding 2–3 drops of 15% KI solution and 5 drops of 1% starch solution) was titrated with 0.001 n KIO_3 solution to a light blue colour, stable for 60 s.

For all analyses, the repeatability was trifold. The results were compared on the Tukey's mean separation test at a significance level of $P \leq 0.05$ and were processed by Pearson's correlation analysis using Minitab 19 software (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

The results of our research showed that the content of soluble solids in sweet cherry fruits ranged from 11.89 to 19.56% under bare fallow conditions and from 14.15 to 19.99% under living mulch conditions (Tables 1 and 2). Soluble solids content was significantly higher under living mulch conditions compared to bare fallow: by 19% in 2018 in the stage of petal fall, by 18% in the stage of partial reddening of fruits; in 2019 in the stages of petal fall, hardening of the stone and partial reddening of the fruits - respectively by 19, 20 and 18%. During fruit ripening, the content of soluble solids in the fruits gradually increased and became the highest in the stage of picking ripeness. Correlation analysis showed a strong direct positive correlation between soluble solids content and the content of sugars, phenolic substances and anthocyanins in sweet cherry fruits in both 2018 and 2019 (Table 3). In 2019, compared to 2018, the content of soluble solids decreased by 6% under the conditions of living mulch, by 12% - under bare fallow conditions.

Sugar content in sweet cherry fruits increased during ripening and ranged from 9.43 to 14.08% for bare fallow orchard floor management system and from 11.22 to 15.05% for living mulch. During the stages of petal fall and stone hardening during the two years of research and partial reddening of the fruits in 2019, a significant difference remained between the variants of the experiment - sugar content in the fruits was significantly higher for living mulch (by 18–37%). However, in the stage of picking ripeness, no significant difference in sugar content in the fruits between the variants of the experiment was observed (although in the conditions of living mulch this index tended to increase). The content of sugars in the fruits correlated the most with the content of phenolic substances and anthocyanins. In 2019, compared to 2018, the content of sugars in cherries in bare fallow conditions remained at the same level, under living mulch conditions - increased by 6%.

Table 1. Phytochemical composition of sweet cherry fruits under different orchard floor management systems in 2018, $\overline{M} \pm m$

Variant	SS, %	Sug, %	TA, %	Phen., mg 100 g^{-1}	Ant., mg 100 g^{-1}	AsA, g 100 g^{-1}	Glu., mg 100 g^{-1}	TRA, mL KIO_3 100 g^{-1}
Petal fall								
Bare fallow	13.20 ± 0.54	9.43 ± 0.24	1.01 ± 0.02	3.4 ± 0.11	0	10.1 ± 0.21	23.0 ± 0.15	7.5 ± 0.04
Living mulch	15.71 ± 0.33*	11.22 ± 0.27*	1.23 ± 0.02*	5.3 ± 0.12*	0	12.1 ± 0.28*	26.1 ± 0.22*	8.5 ± 0.03*
Hardening of the stone								
Bare fallow	14.05 ± 0.57	10.01 ± 0.39^{a}	0.83 ± 0.03^{a}	18.7 ± 0.35^{a}	0	15.4 ± 0.34^{a}	27.6 ± 0.34^{a}	9.1 ± 0.18^{a}
Living mulch	16.80 ± 0.69	12.22 ± 0.35*^{a}	1.05 ± 0.02*^{a}	26.4 ± 0.38*^{a}	0	17.6 ± 0.35*^{a}	29.2 ± 0.25*^{a}	9.5 ± 0.20^{a}
Partial reddening								
Bare fallow	15.90 ± 0.44^{a}	11.36 ± 0.37^{a}	0.78 ± 0.02^{a}	59.1 ± 0.43^{a}	4.93 ± 0.04	7.9 ± 0.48^{a}	23.8 ± 0.53^{a}	7.8 ± 0.08^{a}
Living mulch	18.76 ± 0.49*^{a}	13.40 ± 0.43*^{a}	0.92 ± 0.03^{a}	75.8 ± 0.44*^{a}	5.95 ± 0.03*	9.5 ± 0.49*^{a}	24.6 ± 0.41^{a}	8.0 ± 0.09
Picking ripeness								
Bare fallow	19.56 ± 0.67^{a}	13.93 ± 1.21^{a}	0.64 ± 0.06^{a}	67.1 ± 0.72^{a}	7.36 ± 0.04^{a}	7.3 ± 0.58	21.5 ± 0.42^{a}	7.2 ± 0.09^{a}
Living mulch	19.99 ± 0.22^{a}	14.25 ± 1.27	0.72 ± 0.07^{a}	98.9 ± 0.80*^{a}	10.12 ± 0.23*^{a}	9.4 ± 0.71*	22.3 ± 0.46^{a}	7.3 ± 0.09^{a}

*– significant difference between the variants at $P \leq 0.05$; ^{a} – significant difference at $P \leq 0.05$ in comparison with the previous stage of organogenesis. Phen: phenolic substances, GA: gallic acid.

Table 2. Phytochemical composition of sweet cherry fruits under different orchard floor management systems in 2019, $\overline{M} \pm m$

Variant	SS, %	Sug, %	TA, %	Phen., mg 100 g^{-1}	Ant., mg 100 g^{-1}	AsA, mg 100 g^{-1}	Glu., mg 100 g^{-1}	TRA, mL KIO_3 100 g^{-1}
Petal fall								
Bare fallow	11.89 ± 0.55	9.52 ± 0.46	1.09 ± 0.03	4.1 ± 0.23	0	13.6 ± 0.33	15.4 ± 0.23	5.2 ± 0.14
Living mulch	14.15 ± 0.54*	11.33 ± 0.48*	1.30 ± 0.03*	6.4 ± 0.29*	0	13.2 ± 0.35	17.9 ± 0.22*	6.1 ± 0.24*
Hardening of the stone								
Bare fallow	12.61 ± 0.68	10.11 ± 0.69	0.90 ± 0.02^{a}	22.8 ± 0.45^{a}	0	16.8 ± 0.34^{a}	19.0 ± 0.16^{a}	6.5 ± 0.45^{a}
Living mulch	15.14 ± 0.74*	12.12 ± 0.65*	1.08 ± 0.04*^{a}	32.1 ± 0.48*^{a}	0	19.4 ± 0.56*^{a}	22.1 ± 0.21*^{a}	7.2 ± 0.39*^{a}
Partial reddening								
Bare fallow	14.32 ± 0.85^{a}	11.47 ± 0.64	0.84 ± 0.02^{a}	72.7 ± 0.65^{a}	7.65 ± 0.38	10.6 ± 0.32^{a}	22.4 ± 0.53^{a}	7.3 ± 0.21^{a}
Living mulch	16.90 ± 0.91*^{a}	13.53 ± 0.71*	1.02 ± 0.03*^{a}	92.4 ± 0.59*^{a}	9.21 ± 0.35*	10.3 ± 0.58^{a}	24.5 ± 0.57^{a}	7.5 ± 0.24
Picking ripeness								
Bare fallow	17.21 ± 0.67^{a}	14.08 ± 1.14	0.73 ± 0.06^{a}	89.3 ± 0.72^{a}	12.05 ± 0.19a	8.4 ± 0.59^{a}	20.4 ± 0.55^{a}	6.7 ± 0.47^{a}
Living mulch	18.75 ± 1.18^{a}	15.05 ± 1.18	0.75 ± 0.04^{a}	110.1 ± 0.75*^{a}	15.23 ± 0.33*^{a}	10.2 ± 0.87*	21.5 ± 0.61^{a}	7.2 ± 0.55^{a}

*– significant difference between the variants at $P \leq 0.05$; ^{a} – significant difference at $P \leq 0.05$ in comparison with the previous stage of organogenesis. Phen: phenolic substances, GA: gallic acid.

Organic acids affect the taste of fruits and perform several metabolic functions, including maintaining turgor pressure (Famiani et al., 2015; Walker et al., 2018), play a role in fruit ripening, absorbing Ca from pectins, which makes pectins soluble and thus help soften the fruits (Richter, 2001, p. 29). The content of titrated acids in sweet cherry fruits decreased during fruit ripening, reaching a minimum in the phase of picking ripeness and in bare fallow conditions ranged from 1.09 to 0.64%, in living mulch conditions - from 1.30 to 0.72%. During the stages of petal fall and stone hardening in 2018 and during the petal fall, stone hardening and partial fruit reddening phases in 2019, titrated acids content in fruits was significantly higher under the conditions of living mulch (by 20, 37, 18, 22 and 25%, respectively). In the phase of picking ripeness, the difference between the variants of the experiment was statistically insignificant both in 2018 and in 2019. The content of titrated acids in fruits mostly correlated with the content of phenolic substances and anthocyanins. In 2019, compared to 2018, the content of titrated acids in fruits increased by 17% under the conditions of bare fallow, by 14% - under living mulch conditions. There are reports in the scientific literature that the content of organic acids in sweet cherry fruits increases in the process of their ripening (Serrano et al., 2005; Tahir et al., 2013). In our study, however, the content of titrated acids in sweet cherry fruits decreased during ripening. It should be noted that the content of organic acids in the fruits was relatively low in our study. Probably, this is due to the peculiarities of the climatic conditions of our region and the peculiarities of the studied cultivar. After all, the content of

Table 3. Correlation coefficients (r^2) between the content of biologically active substances in sweet cherry fruits

Correlation coefficients between:	Year of the research	
	2018	2019
Soluble solids and sugars	0.99**	0.99**
Soluble solids and titrated acids	0.47*	0.27^{ns}
Soluble solids and ascorbate	0.28^{ns}	0.62*
Soluble solids and phenolic substances	0.51*	0.82**
Soluble solids and anthocyanins	0.74*	0.81**
Soluble solids and glutathione	0.11^{ns}	0.65*
Sugars and titrated acids	0.45*	0.31^{ns}
Sugars and ascorbate	0.28^{ns}	0.67*
Sugars and phenolic substances	0.78**	0.79**
Sugars and glutathione	0.57*	0.47*
Titrated acids and ascorbate	0.32^{ns}	0.51*
Titrated acids and phenolic substances	0.51*	0.61*
Titrated acids and glutathione	0.46*	0.21^{ns}
Ascorbate and phenolic substances	0.40*	0.72**
Ascorbate and glutathione	0.76**	0.36*
Phenolic substances and glutathione	0.27^{ns}	0.56*
Anthocyanins and sugars	0.72**	0.93**
Anthocyanins and titrated acids	0.58*	0.59*
Anthocyanins and ascorbate	0.54*	0.72*
Anthocyanins and phenolic substances	0.95**	0.95**
Anthocyanins and glutathione	0.36*	0.32*

Notes: **Correlation is significant at 0.01 levels; *Correlation is significant at 0.05 levels; ns correlation is not significant.

titrated acids in the fruits depends significantly on the characteristics of the sweet cherry cultivar (Corneanu et al., 2020). It has been reported that in arid and hot conditions during fruit ripening, the content of organic acids in sweet cherry fruits decreases (Pangelova, 1970; Skvareninova, 1997; Richter, 2001, pp. 34–36; Famiani et al., 2020): the acid content decreases during fruit ripening, as they are spent on energy and plasticity metabolism during intensive mesocarp growth (organic acid respiration). Regarding the decrease in the content of soluble solids, sugars, titrated acids in the fruits under bare fallow conditions, compared with living mulch, this is partly due to the tendency to increased fruit weight under bare fallow (Gerasko, 2020), as it was reported that increasing fruit weight may reduce the content of soluble solids, sugars, titrated acids, leukoanthocyanidins - there is a so-called 'dilution' due to an increase in cell volume (Richter, 2001, p. 37; Famiani et al., 2015; Walker et al., 2018; Famiani et al., 2020).

The total content of phenolic compounds in sweet cherry fruits was in the range of 3.4–89.3 mg of GA 100 g^{-1} of raw mass under the conditions of bare fallow and significantly higher (by 23–56% during all stages of fruit ripening) under the conditions of living mulch - from 5.3 to 110.1 mg of GA 100 g^{-1} of raw mass. Phenolic substances accumulated in the fruits during ripening, reaching a maximum in the stage of picking ripeness, during which in 2018 they were 47% higher under the conditions of living mulch, compared to the conditions of bare fallow, in 2019 - 23% higher. The content of phenolic substances correlated mostly with the content of anthocyanins. This is natural, as anthocyanins are the most represented class of phenolic compounds in red-coloured sweet cherry fruits (Martini et al., 2017). Compared to 2018, in 2019 the content of phenolic substances in the fruits increased by 33% under bare fallow conditions, and by 11% - under living mulch conditions. Phenolic substances, as a dietary component, play an important role in shaping the sensory characteristics of fruits, giving them specific tartness, as well as being responsible for their colour and firmness (Richter, 2001, p. 38). Phenolic substances are natural antioxidants with a strong ability to neutralize free radicals; they exhibit anti-cancer, anti-ulcer properties (Ballistreri et al., 2013). The composition and content of phenolic substances in fruits depends, not least, on cultivar, genotype, climatic and agronomic conditions (Vursavus et al., 2006; Średnicka-Tober et al., 2019).

Based on our results, living mulch promotes phenolic substance accumulation in sweet cherry fruits, which makes them especially valuable for therapeutic and prophylactic nutrition.

Anthocyanin content in sweet cherry fruits was in the range from 0 to 12.1 mg 100 g^{-1} of raw mass under the conditions of bare fallow and from 0 to 15.2 mg 100 g^{-1} of fresh mass under the conditions of living mulch. Anthocyanins accumulated in fruits starting from the stage of partial reddening, with significantly more anthocyanins contained in the fruits under living mulch conditions, both in 2018 (by 36%) and in 2019 (by 26%). In 2019, compared to 2018, more anthocyanins accumulated in the fruits: by 63% under the conditions of bare fallow, and by 51% under the conditions of living mulch. The increase in anthocyanin content in the fruits is due to their participation in the formation of the characteristic colour of the fruit.

Weather conditions significantly affect anthocyanin content in sweet cherry fruits: in dry and hot weather, the content of anthocyanins increases (Goncalves et al., 2004).

The content of ascorbic acid in the fruits reached a maximum in the stage of the hardening of the stone (15.4–16.8% under bare fallow and 17.6–19.4% under living mulch), and then decreased during fruit ripening, but in the stage of picking ripeness was significantly higher in living mulch conditions compared to bare fallow conditions (by 29% in 2018 and by 21% in 2019). The greatest correlation was observed between the content of ascorbate and the content of phenolic substances, glutathione and anthocyanins. In 2019, compared to 2018, ascorbate content increased by 15% in bare fallow conditions, by 9% - in living mulch conditions. Ascorbate (vitamin C) is the most well-known antioxidant for consumers, which plays a protective role against cardio-vascular diseases (Vilchèze et al., 2018; Wang et al., 2018). Therefore, it can be stated that sweet cherry fruits grown in living mulch conditions have a higher consumer quality compared to fruits grown under bare fallow.

Glutathione content in the fruits reached a maximum in the stage of hardening of the stone in 2018 and in the phase of partial reddening of the fruits in 2019. Under living mulch conditions, the content of glutathione in the fruits was significantly higher compared to the conditions of bare fallow in the stages of petal fall and hardening of the stone (in 2018, respectively, 18 and 14%, in 2019 – 19 and 11%). Glutathione content in 2019, compared to 2018, has not changed in both variants of the experiment. Vitamin C (l-ascorbate, l-ascorbic acid) and glutathione are the main hydrophilic antioxidants in plants, which play an important role in plant stress resistance and fruit quality (Davey & Keulemans, 2004; Díaz-Mula et al., 2009b; Foyer & Noctor, 2011). Therefore, the increase in the content of ascorbate and glutathione in sweet cherry fruits under the conditions of living mulch, compared with bare fallow, indicates increased stress resistance and higher functional quality of such fruits for consumption.

The overall reducing activity tended to increase under living mulch conditions and was significantly higher compared to bare fallow: in the stage of petal fall in 2018 (by 13%), in the phases of falling petals and hardening of the bone in 2019, respectively, by 17 and 11%. In 2019, compared to 2018, the overall reducing activity remained virtually unchanged in both versions of the experiment. The highest total reducing activity correlated with the content of glutathione, ascorbate and dry soluble substances (Table 4). Our data is consistent with the results of studies by Commisso (2017): phenolic compounds are the main source of antioxidant activity, and experiments with artificial simplified phytocomplexes have shown strong synergies between anthocyanins and ascorbic acid.

Table 4. Correlation coefficients (r^2) of total reducing activity (TRA) with the content of biologically active substances in sweet cherry fruits

Year of the research	Correlation coefficient of TRA with the content:						
	SS	Sug	TA	Phen	Ant	AsA	Glu
2018	0.52**	0.25^{ns}	0.34*	0.32*	0.53**	0.88**	0.90*
2019	0.64**	0.49*	0.20^{ns}	0.79*	0.32*	0.36*	0.95*

Notes: **Correlation is significant at 0.01 levels; *Correlation is significant at 0.05 levels; ns correlation is not significant. SS: Soluble solids; Sug: Sugars; TA: Titrated acids; Phen: Phenolic substances; Ant: Anthocyanins; AsA: Ascorbate, Glu: Glutathione.

It should be noted that sweet cherry fruits grown organically in our experiment, had high phytochemical characteristics compared to the fruits grown in intensive technology in the region (Tolstolik, 2016; Bondarenko, 2018). The data on biochemical composition of sweet cherry fruits obtained by us coincides with the average data obtained in the

South of Ukraine (Richter, 2001; Kishchak, 2012; Ivanova et al., 2021). Anthocyanins content in sweet cherry fruits in our research is generally consistent with the data obtained by Italian researchers (Ballistreri et al., 2013), but is significantly lower compared to Spanish studies (González-Gómez et al., 2010). The total content of polyphenols in fruits in our study (during the ripening stage) is significantly lower, and the sugar content is consistent with data obtained for old sweet cherry cultivars in Czech Republic (Nawirska-Olszańska et al., 2017). It should be noted that as of today, there are new sweet cherry cultivars that are characterized by high anthocyanins content (approximately three times more than 'Dilemma' cultivar we studied) (Antognoni et al., 2020). If we compare phytochemical composition of sweet cherry fruits grown in our research area (South of Ukraine) with fruits grown in Turkey (Kelebek & Selli, 2011), 'Dilemma' fruits contain significantly more sugars, significantly less titrated acids, and the total phenolic content in them is similar to the level in 'Van' cultivar. Content of sugars, titrated acids and anthocyanins in 'Dilemma' fruits during the ripening stage was similar to the content of these compounds in the fruits of 'Bianca' sweet cherry grown in southern Poland (Skrzyński et al., 2016).

In our study, the content of soluble solids, sugars, phenolic substances and anthocyanins increased in sweet cherry fruits as they ripened and was closely correlated with the overall reducing activity. Similar trends have already been noted in the works by O.A. Grebennikova (2011): the content of biologically active substances in plum fruits increased during ripening and was greatest in mature and even overripe fruits, while the correlation between antioxidant activity and the content of biologically active substances also increased as the fruits ripened; H. M. Díaz-Mula et al. notes (2009a): as sweet cherry fruits mature, the nutritional and functional quality of them increases (including the content of phenols, anthocyanins and antioxidant capacity).

In our study, both variants were grown using organic technology. But sweet cherry trees grown under living mulch orchard floor management system experienced additional stress from competing with grasses. This contributed to the accumulation of protective antioxidant and biologically active compounds in the fruits. Fruits enriched with endogenous antioxidants are in good demand from consumers and retain consumer and functional quality for longer (have a longer shelf life) (Serrano et al., 2009). Therefore, it can be stated that using living mulch in the orchard helps to improve the consumer and functional quality of sweet cherry fruits.

CONCLUSIONS

The content of ascorbate, phenolic substances and anthocyanins in sweet cherry fruits under living mulch conditions were consistently significantly higher compared to the fruits under bare fallow conditions. Identified patterns can be explained by the stressful conditions of competition with natural grasses, which activates the synthesis of anti-stress biologically active substances in plant tissues (including fruits).

Since it is the antioxidants of the fruits that have physiological value for human health, it can be stated that fruits grown in the conditions of living mulch have a higher therapeutic and prophylactic value than the fruits grown in the conditions of bare fallow.

The content of dry soluble substances, sugars, titrated acids, glutathione and total reducing activity in the fruits experienced significant fluctuations during fruit ripening, but during the stage of picking maturity there was no significant difference between the variants of the experimental both in 2018 and 2019.

REFERENCES

Anjum, N.A., Umar, S. & Chan, M.T. 2010. *Ascorbate-Glutathione Pathway and Stress Tolerance in Plants.* Springer Dordrecht Heidelberg, London New York. https://doi.org/10.1007/978-90-481-9404-9

Antognoni, F., Potente, G., Mandrioli, R., Angeloni, C., Freschi, M., Malaguti, M. & Tartarini, S. 2020. Fruit Quality Characterization of New Sweet Cherry Cultivars as a Good Source of Bioactive Phenolic Compounds with Antioxidant and Neuroprotective Potential. *Antioxidants* **9**, 677. https://doi.org/10.3390/antiox9080677

Atucha, A., Merwin, I.A. & Brown, M.G. 2011. Long-term effects of four groundcover management systems in an apple orchard. *HortSci* **46**(8), 1176–1183. https://doi.org/10.21273/HORTSCI.46.8.1176

Atucha, A., Merwin, I.A., Brown, M.G., Gardiazabal, F., Mena, F., Adriazola, C. & Lehmann, J. 2013. Soil erosion, runoff and nutrient losses in an avocado (Persea americana Mill) hillside orchard under different groundcover management systems. *Plant Soil* **368**(1–2), 393–406. https://doi.org/10.1007/s11104-012-1520-0

Ballistreri, G., Continella, A., Gentile, A., Amenta, M., Fabroni, S. & Rapisarda, P. 2013. Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. *J. Food Chem.* **140**, 630–638. https://doi.org/10.1016/j.foodchem.2012.11.024

Bondarenko, P. 2018. Influence of rootstock-scion combinations and conditions of the year on sweet cherry fruit quality in the conditions of the Southern Steppe of Ukraine. *Bulletin of Lviv National Agrarian University. Agronomy* **22**(2), 96–102. https://doi:10.31734/agronomy2018.02.096 (in Ukrainian)

Commisso, M. 2017. Multi-approach metabolomics analysis and artificial simplified phytocomplexes reveal cultivar-dependent synergy between polyphenols and ascorbic acid in fruits of the sweet cherry (Prunus avium L.). *PLoS ONE* **12**(7), e0180889. https://doi.org/10.1371/journal.pone.0180889

Corneanu, M., Iurea, E. & Sîrbu, S. 2020. Biological properties and fruit quality of sweet cherry (Prunus avium L.) cultivars from Romanian assortment. *Agronomy Research* **18**(4), 2353–2364. doi:10.15159/ar.20.231

Culumber, C.M., Reeve, J.R., Black, B.L., Ransom, C.V. & Alston, D.G. 2019. Organic orchard floor management impact on soil quality indicators: nutrient fluxes, microbial biomass and activity. *Nutr Cycl Agroecosyst* **115**, 101. https://doi.org/10.1007/s10705-019-10007-2

Davey, M.W. & Keulemans, J. 2004. Determining the potential to breed for enhanced antioxidant status in Malus: mean inter- and intravarietal fruit vitamin C and glutathione contents at harvest and their evolution during storage. *J. Agric. Food Chem.* **52**, 8031–8038. https://doi.org/10.1021/jf048531k

Díaz-Mula, H.M., Castillo, S., Martínez-Romero, D., Valero, D., Zapata, P.J., Guillén, F. & Serrano, M. 2009a. Sensory, Nutritive and Functional Properties of Sweet Cherry as Affected by Cultivar and Ripening Stage. *Food Science and Technology International* **15**(6), 535–543. https://doi.org/10.1177/1082013209351868

Díaz-Mula, H., Valero, D., Zapata, P., Guillén, F., Castillo, S., Martínez-Romero, D. & Serrano, M. 2009b. The functional properties of sweet cherry as a new criterion in a breeding program. *Acta Hort* **839**, 275–280. https://doi.org/10.17660/ActaHortic.2009.839.34

Famiani, F, Bonghi, C, Chen, Z.-H., Drincovich, M.F., Farinelli, D., Lara, M.V. & Walker, R.P. 2020. Stone Fruits: Growth and Nitrogen and Organic Acid Metabolism in the Fruits and Seeds. *Plant Sci.* **11**, 572601. https://doi.org/10.3389/fpls.2020.572601

Famiani, F., Battistelli, A., Moscatello, S., Cruz-Castillo, J.G. & Walker, R.P. 2015. The organic acids that are accumulated in the flesh of fruits: occurrence, metabolism and factors affecting their contents - a review. *Rev. Chapingo Ser. Hortic* **21**, 97–128. https://doi.org/10.5154/r.rchsh.2015.01.004

Fidalski, J., Tormena, C.A. & da Silva, A.P. 2010. Least limiting water range and physical quality of soil under groundcover management systems in citrus. *Sci. agric. (Piracicaba, Braz.)* **67**(4). https://doi.org/ 10.1590/S0103-90162010000400012

Fonseca, L.R.S., Silva, G.R., Luís, Â., Cardoso, H.J., Correia, S., Vaz, C.V. & Socorro, S. 2021. Sweet Cherries as Anti-Cancer Agents: From Bioactive Compounds to Function. *Molecules* **26**, 2941. https://doi.org/10.3390/molecules26102941

Foyer, C.H. & Noctor, G. 2011. Ascorbate and Glutathione: The Heart of the Redox Hub. *Plant Physiology* **155**(1), 2–18. https://doi.org/10.1104/pp.110.167569

Francis, F.J. 1982. Analysis of anthocyanins. In P. Markakis (Ed.), *Anthocyanins as food colors*. Academic Press, New York, NY, 181–207. https://doi.org/10.1016/B978-0-12-472550-8.50011-1

Ivanova, I., Serdyuk, M., Malkina, V., Priss, O., Herasko, T. & Tymoshchuk, T. 2021. Investigation into sugars accumulation in sweet cherry fruits under abiotic factors effects. *Agronomy Research* **19**(2), 444–457. doi:10.15159/ar.21.004

Gerasko, T.V. 2020. Influence of live mulch on physiological and biochemical parameters of cherry leaves and fruits by organic cultivation technology. *Collection of scientific works "Agrobiology"* **1**, 20–28. https://doi.org/10.33245/2310-9270-2020-157-1-20-28 (in Ukrainian).

Gerasko, T., Pyda, S. & Ivanova, I. 2021. Effect of Living Mulch on Soil Conditions and Morphometrical Indices of Sweet Cherry Trees. *Inter J Appl Agric Sci* **7**(1), 50–56. https://doi.org/10.11648/j.ijaas.20210701.14

Gomez, J.A., Amato, M., Celano, G. & Koubouris, G. C. 2008. Organic olive orchards on sloping land: more than a specialty niche production system? *J. Environ Sci.* **89**(2), 99–109. https://doi.org/10.1016/j.jenvman.2007.04.025

Gonçalves, A.C., Bento, C., Silva, B., Simões, M. & Silva, L.R. 2019. Nutrients, Bioactive Compounds and Bioactivity: The Health Benefits of Sweet Cherries (*Prunus avium* L.) *Current Nutrition & Food Science* **15**(3), 208–227. https://doi.org/10.2174/1573401313666170925154707

Goncalves, B., Landbo, A.-K., Knudsen, D., Silva, A. P., Moutinho-Pereira, J., Rosa, E. & Meyer, A. S. 2004. Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). *J. Agric. Food Chem.* **52**, 523–530. https://doi.org/10.1021/jf030595s

González-Gómez, D., Lozano, M., Fernández-León, M.F., Bernalte, M.J., Ayuso, M.C. & Rodríguez, A. B. 2010. Sweet cherry phytochemicals: Identification and characterization by HPLC-DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain). *Journal of Food Composition and Analisis* **23**(6), 533–539. https://doi.org/10.1016/j.jfca.2009.02.008

Gorodniy, M.M., Melnichuk, S.D. & Gonchar, O.M. 2006. *Applied biochemistry and quality management of crop products: Textbook*. Aristei, Kyiv, 484 pp., p. 442–443 (in Ukrainian).

Grebennikova, O.A. 2011. *Biologically active substances in fruits, leaves and products of processing of perspective varieties of plum*. PhD Thesis, Odessa, 22pp. (in Ukrainian).

Gu, C., Liu, Y., Mohamed, I., Zhang, R., Wang, X., Nie, X. & Li, Z. 2016. Dynamic Changes of Soil Surface Organic Carbon under Different Mulching Practices in Citrus Orchards on Sloping Land. *PLoS ONE* **11**(12), e0168384. https://doi.org/10.1371/journal.pone.0168384

Hallmann, E. & Rozpara, E. 2017. The estimation of bioactive compounds content in organic and conventional sweet cherry (Prunus avium L.). *J. Res. Appl. Agric. Eng.* **62**, 141–145. http://yadda.icm.edu.pl/baztech/element/bwmeta1.element.baztech-584e09be-6f70-4b88-a129-5c0ba9ff7254

Holden, E., Linnerud, K., Banister, D., Schwanitz, V.J. & Wierling, A. 2017. *The Imperatives of Sustainable Development: Needs, Justice, Limits.* Routledge, London, UK. https://doi.org/10.4324/9780203022177

Kelebek, H. & Selli, S. 2011. Evaluation of chemical constituents and antioxidant activity of sweet cherry (Prunus avium L.) cultivars. *Int J Food Sci Technol* **46**, 2530–2537. https://doi.org/10.1111/j.1365-2621.2011.02777.x

Kishchak, O.A. 2012. Commodity quality and biochemical composition of cherry fruits depending on the type of plantings. *Bulletin of Agricultural Science* **4**, 37–41. Retrieved from http://nbuv.gov.ua/UJRN/vaan_2012_4_7 (in Ukrainian)

Martini, S., Conte, A. & Tagliazucchi, D. 2017. Phenolic compounds profile and antioxidant properties of six sweet cherry (Prunus avium) cultivars. *Food Research International* **97**, 15–26. https://doi.org/10.1016/j.foodres.2017.03.030

Merwin, I.A. 2010. Keeping Under Cover: The Ideal Look of an Orchard Floor. *Fruit Growers News.* Retrieved from http://fruitgrowersnews.com/article/keeping-under-cover-the-ideal-look-of-an-orchard-floor/

Methods of qualification examination of plant varieties for suitability for distribution in Ukraine. *Methods for determining the quality of crop products*. 2021. Retrieved from https://minagro.gov.ua/ua/napryamki/roslinnictvo/reyestr-sortiv-roslin-ukrayini/metodiki-provedennya-kvalifikacijnoyi-ekspertizi-sortiv-roslin (in Ukrainian).

Nawirska-Olszańska, A., Kolniak-Ostek, J., Oziembłowski, M., Ticha, A., Hyšpler, R., Zadak, Z. & Paprstein, F. 2017. Comparison of old cherry cultivars grown in Czech Republic by chemical composition and bioactive compounds. *Food Chem* **228**, 136–142. https://doi.org/10.1016/j.foodchem.2017.01.154

Neilsen, G.H., Neilsen, D., Kappel, F. & Forge, T. 2014. Interaction of Irrigation and Soil Management on Sweet Cherry Productivity and Fruit Quality at Different Crop Loads that Simulate Those Occurring by Environmental Extremes. *HortScience* **49**(2), 215–220. https://doi.org/10.21273/HORTSCI.49.2.215

Palese, A.M. 2014. Influence of soil management on soil physical characteristics and water storage in a mature rainfed olive orchard. *Soil Tillage Res* **144**, 96–109. https://doi.org/10.1016/j.still.2014.07.010

Pangelova, J. 1970. Chemical composition of the fruits of some cherry and sour cherry varieties. *Fruit growing* **8**, 30–33. (in Bulgarian)

Richter, A.A. 2001. *Improving the quality of fruits of southern cultures*. Tavria, Simferopol. 426 pp. (in Russian).

Sánchez, E.E., Giayetto, A., Cichón, L., Fernández, D., Aruani, M.C. & Curetti, M. 2007. Cover crops influence soil properties and tree performance in an organic apple (*Malus domestica* Borkh) orchard in northern Patagonia. *Plant Soil* **292**(1–2), 193–203. https://doi.org/10.1007/s11104-007-9215-7

Serrano, M., Guillén, F., Martínez-Romero, D., Castillo, S. & Valero, D. 2005. Chemical Constituents and Antioxidant Activity of Sweet Cherry at Different Ripening Stages. *J. Agric. Food Chem* **53**(7), 2741–2745. https://doi.org/10.1021/jf0479160

Serrano, M., Diaz-Mula, H. M., Zapata, P.J., Castillo, S., Guillen, F., Martinez-Romero, D. & Valero, D. 2009. Maturity stage at harvest determines the fruit quality and antioxidant potential after storage of sweet cherry cultivars. *J. Agric. Food Chem.* **57**, 3240–3246. https://doi.org/10.1021/jf803949k

Simões, M.P., Belo, A., Cruz, C.P. & Pinheiro, A.C. 2014. Natural vegetation management to conserve biodiversity and soil water in olive orchards. *Spanish J. Agric. Res.* **12**(3), 633–643. https://doi.org/10.5424/sjar/2014123-5255

Skrzyński, J., Leja, M., Gonkiewicz, A. & Banach, P. 2016. Cultivar effect on the sweet cherry antioxidant and some chemical attributes. *Folia Hort* **28**(1), 95–102. https://doi.org/10.1515/fhort-2016-0011

Skvareninova, J. 1997. Premenlivost Kvality Populacii Cerasne Vtacej (Ctrasus avium (L.) Moench.) a jej vertikalne rozsirenie v niektorych oblastiach Slovenska. *Acta Fac Forest Zvolen* **39**, 21–31. (in Slovak)

Średnicka-Tober, D., Ponder, A., Hallmann, E., Głowacka, A. & Rozpara, E. 2019. The Profile and Content of Polyphenols and Carotenoids in Local and Commercial Sweet Cherry Fruits (Prunus avium L.) and Their Antioxidant Activity In Vitro. *Antioxidants* **8**(11), 534. https://doi.org/10.3390/antiox8110534

Tahir, M., Farooq, A., Bhatti, I.A. & Tahira, I. 2013. Effect of maturity on proximate composition, phenolics and antioxidant attributes of cherry fruit. *Pakistan Journal of Botany* **45**(3), 909–914. https:http://www.pakbs.org/.../25.pdf

Tolstolik, L. 2016. *Biochemical composition and technological properties of fruits of elite forms and varieties of cherries.* Retrieved from http://sophus.at.ua/publ/2016_10_28_kampodilsk/sekcija_section_1_2016_10_28/biokhimichnij_sklad_i_tekhnologichni_vlastivosti_plodiv_elitnikh_form_ta_sortiv_chereshni/129-1-0-2013 (in Ukrainian).

Vilchèze, C., Kim, J. & Jacobs, W.R., Jr. 2018. Vitamin C potentiates the killing of Mycobacterium tuberculosis by the first-line tuberculosis drugs isoniazid and rifampin in mice. *Antimicrob Agents Chemother* **62**, e02165-17. https://doi.org/10.1128/AAC.02165-17

Vursavus, K., Kelebek, H. & Selli, S. 2006. A study on some chemical and physico-mechanic properties of three sweet cherry varieties (Prunus avium L.) in Turkey. *J. Food Eng.* **74**, 568–575. https://doi.org/10.1016/j.jfoodeng.2005.03.059

Walker, R.P., Benincasa, P., Battistelli, A., Moscatello, S., Técsi, L., Leegood, R.C. & Famiani, F. 2018. Gluconeogenesis and nitrogen metabolism in maize. *Plant Physiol Biochem* **130**, 324–333. https://doi.org/10.1016/j.plaphy.2018.07.009

Wang, K., Jiang, H., Li, W., Qiang, M., Dong, T. & Li, H. 2018. Role of vitamin C in skin diseases. *Front Physiol* **9**, 819. https://doi.org/10.3389/fphys.2018.00819

Workshop on agrochemistry: *Textbook.* / Ed. V.G. Mineev. 2001. MSU Publishing House, Moscow, 689 pp., p. 419–422 (in Russian).

Yaman, M. 2022a. Evaluation of genetic diversity by morphological, biochemical and molecular markers in sour cherry genotypes. *Molecular Biology Reports* **49**(6), 5293–5301. https://doi.org/10.1007/s11033-021-06941-6

Yaman, M. 2022b. Determination of genetic diversity in european cranberrybush (Viburnum opulus L.) genotypes based on morphological, phytochemical and ISSR markers. *Genetic Resources and Crop Evolution* **69**(5), 1889–1899. https://doi.org/10.1007/s10722-022-01351-4

Yao, S., Merwin, I.A., Bird, G.W., Abawi, G.S. & Thies, J.E. 2005. Orchard floor management practices that maintain vegetative or biomass groundcover stimulate soil microbial activity and alter soil microbial community composition. *Plant Soil.* **271**(1–2), 377–389. https://doi.org/10.1007/s11104-004-3610-0

Zheng, W., Gong, Q., Zhao, Z., Liu, J., Zhai, B., Wang, Z. & Li, Z. 2018. Changes in the soil bacterial community structure and enzyme activities after intercrop mulch with cover crop for eight years in an orchard. *Eur J. Soil Biol.* **86**, 34–41. https://doi.org/10.1016/j.ejsobi.2018.01.009