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THE EFFECT OF ORGANIC PRACTICES ON THE BIOACTIVE COMPOUNDS CONTENT IN STRAWBERRY FRUITS

Summary

In organic agriculture only natural methods of cultivation (fertilization and plant protection) are allowed. The total conventional strawberry production in 2014 was 216 thousand tones on the 53 thousand ha. Organic production was on a very low level with only 5 thousand tones cultivated on 1.3 thousand ha of area. Experiment was carried out in the years2013-2014. One strawberry cultivar Honeyoe was cultivated in two experimental organic farms. In one organic farm EM, as well as in the second only organic practice without effective organisms have been used. Three times of harvest have been organized in every experimental year. In fruits the content of dry matter (by scale method), vitamin C (by spectrophotometric method) as well as polyphenols (by HPLC method) have been determinated. The obtained results were statistically elaborated with post-hoc Tukey test (□=0,05). The obtained results showed that using of effective organisms had significant impact on the caffeic, p-coumaric, quercetin, glucoside-3-O-quercetin and luteolin content in strawberry fruits. The third fruits harvest time was the most effective in case of dry matter, vitamin C, gallic, ellagic, p-coumaric acids as well as some flavonoids (quercetin and its derivates), luteolin, kaempferol and pelargonidine-3,5-di-rutinoside in experimental strawberry fruits.
***Key words**: strawberry, organic cultivation, conventional cultivation, polyphenols, vitamin C*

WPŁYW EKOLOGICZNYCH METOD UPRAWY NA ZAWARTOŚC ZWIĄZKÓW BIOLOGICZNIE CZYNNYCH W OWOCACH TRUSKAWKI

Streszczenie

W rolnictwie ekologicznym dozwolone jest stosowanie tylko naturalnych metod upraw (nawożenia i ochrony roślin). W 2014 roku produkcja truskawek z upraw konwencjonalnych wyniosła 216 tys. ton i uprawiane one były na powierzchni 53 tys. ha. Niestety, produkcja truskawek w systemie ekologicznym była znikoma i wyniosła 5 tys. ton zebranych z powierzchni 1.3 tys. ha. Doświadczenie przeprowadzono w latach 2013-2014 w dwóch gospodarstwach ekologicznych uprawiano jedną odmianę truskawki Honeyoe. W jednym z gospodarstw zastosowano efektywne mikroorganizmy, w drugim gospodarstwie ekologicznym nie stosowano żadnych dodatkowych zabiegów agrotechnicznych. Owoce truskawek były zbierane trzy razy w ciągu sezonu owocowania. W dojrzałych owocach oznaczono zawartość: suchej masy (metoda wagową), witaminy C (metoda spektrofotometryczną) oraz polifenoli (metoda HPLC). Otrzymane wyniki zostały poddane analizie statystycznej z użyciem testu post-hoc Tukey'a na poziomie istotności (□=0,05). Wykazały one, że zastosowanie w gospodarstwie ekologicznym efektywnych mikroorganizmów miało istotny wpływ na zawartość kwasu kawowego, p-kumarynowego, kwercetyny i jej glikozydu, jak też luteoliny w owcach truskawek. Najbardziej efektywny okazał się trzeci termin zbioru. Owoce zebrane w tym czasie charakteryzowały się istotnie wyższą zawartością suchej masy, witaminy C, kwasu galusowego, elagowego, p-kumarynowego, jak tez niektórych flawonoidów (kwercetyny i jej pochodnych), luteoliny, kempferolu i rutinozidu-3,5-di-pelargonidyny.
***Słowa kluczowe**: truskawki, uprawa ekologiczna, uprawa konwencjonalna, polifenole, witamina C*

1. Introduction

Effective microorganisms are a composition of bacteria and microorganisms used in time from natural fertilizers, organic fertilizers and compost production. They are used for the soil implementation to create a more favorable microbial environment for growing plants. Better plants condition and nutrition level contributes to better plants growth as well as influences the physiological parameters of the plants. This is reflected in the size, structure and quality of the plant crops [6]. According to the information EM preparation does not contain genetically modified microorganisms, species, only a mix of different other microorganisms. They belong to major families: lactic acid bacteria, yeast and photosynthetic bacterias. EM vaccine starter contains lactic bacteria (*Lactobacillus casei, Streptococcus lactis*), photosynthetic bacterias (*Rhodopseudomonas palustrus, Rhodobacter spae*), yeast (*Saccharomyces album, Candida utilis*), actinomycetes (*Streptomyces album, Streptomyces griseus*), as well as mould (*Aspergillus oryzae, Mucor hiemalis*). Poland is one of the countries with a high production of strawberries, both for fresh market and for processing. The total conventional strawberry production in 2014 was 216 thousand tones on the 53 thousand ha. Organic production was on a very low level with only 5 thousand tones cultivated on 1.3 thousand ha of area [3]. The strawberry fruits are the perfect source of bioactive compounds from different chemical groups: polyphenols, especially phenolic acids (as: chlorogenic and ellagic), flavonoids (as: quercetin and kaempferol and their derivates), as well as anthocyanins, vitamin C [11]. The aim of the work was to compare two organic production systems (with and without effective microorganisms) as well as three harvest times, which stimulated the highest level of bioactive compounds in strawberry fruits.

2. Material and methods

The experiment was carried out in 2013. One strawberry cultivar Honeyoe was cultivated in two experimental organic farms. In one organic farm EM, as well as in the second only organic practice without effective organisms have been used.

Organic farm with used effective microorganisms (with EMO) was located in Głodowo Wielkie, community Nasielsk county nowodworski, Mazovia region. Plantation (0,5 ha of area established in 2012).

Before plants cultivation cow manure as fertilizer have been used in 30 t/ha dose. Following microorganisms preparations have been used:

- EM Farma, in dose of 40 l/ha: after plants re-planting;
- EM Farma Plus, in dose of 20 l/ha: in the middle of April 2013, in the beginning of May 2013 and last time in the beginning of June 2013;
- Ema5, in dose of 20 l/ha: spraying applied three times during vegetation.

In time of harvest plants were weeded by hands.

Organic farm whiteout effective microorganisms were lockated in Płusy, community Obryte, county pułtuski, Mazovia region. Plantation (0,2 ha of area established in 2012). For fertilization following fertilizers have been used: potassium sulfate (allowed in organic agriculture): 0.75 kg/ar. (time: April and May 2013). Plants were weeded four times and mulched by rye straw. The strawberry fruits were collected in three times: on 8 of June, on 15 of June, and on 22 of June in dose 1 kg from each experimental combination. In fruits the content of dry matter (by scale method), vitamin C (by spectrophotometric method) as well as polyphenols (by HPLC method) have been determinated. The obtained results were statistically elaborated with post-hoc Tukey test (□=0,05).

3. Results

The use of effective microorganisms did not have a significant effect on dry matter content and vitamin C in fruits of examined strawberry cultivar Honeyoe (tab. 1). Fruits harvested in the third harvest-time were characterized by significantly highest dry matter content and vitamin C in fruits compared to Ist and IInd time. There were neither effect of effective microorganism influence nor harvest time on the content of total polyphenols in strawberry fruits. Only in the case of total phenolic acid content the positive effect of the microorganism was observed. Strawberry fruits harvested at the first harvest time, were characterized by significantly higher content of phenolic acids in fruit compared to second and third harvest time (Tab. 1). Total flavonoids content was significantly dependent on the applied agricultural practices. When effective microorganisms have been used in the cultivation the fruits were characterized by significantly higher level of total flavonoids in comparison with the cultivation, in which microorganisms have not been applied. Significantly more flavonoids contained fruits collected in the third harvest time and was 7.47 mg/100 g FW. There was neither positive effect of the effective microorganisms influence nor harvest time on the content of total anthocyanins in the test fruit strawberry Honeyoe (Tab. 1).

In the case of individual phenolic acids analysis it was observed the lack of impact of the application of effective microorganisms on chlorogenic and ellagic acid content in strawberry fruits (Fig. 1).

Fruit obtained from plants grown without effective microorganisms contained significantly higher level of gallic acid (2.16 mg/100 g FW), while in the cultivation, with EM fruits contained more caffeic acid and p-coumaric acid. In the case of three phenolic acids (gallic, p-coumaric and ellagic) a significant effect of the harvest time on the content of selected phenolic acids was found.

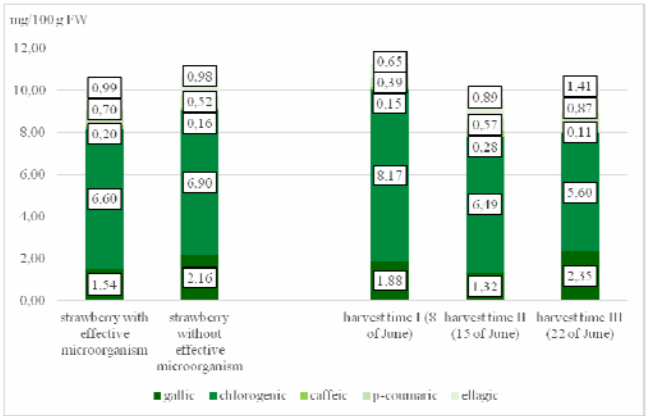

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Fig. 1. The content of identified phenolic acids in experimental strawberry fruits depending on the cultivation method and harvest time

Rys. 1. Zawartość zidentyfikowanych kwasów fenolowych w badanych owocach truskawki w zależności od metody uprawy i czasu zbioru

Table 1. The content of dry matter (g/100 g FW), vitamin C (mg/100 g FW) and total polyphenols, phenolic acids, flavonoids (mg/100 g FW) as well as anthocyanins in examined strawberry fruits

Tab. 1. Zawartość suchej masy (g/100 g ś.m.), witaminy C (mg/100 g ś.m.) oraz polifenoli, kwasów fenolowych, flawonoidów i antocyjanów (mg/100 g ś.m.) w badanych owocach truskawki

Experimental combination	dry matter	vitamin C	total polyphenols	total phenolic acids	total flavonoids	total anthocyanins
strawberry with effective microorganism	10.13±1.88* a	57.88±22.15 a	62.89±20.36 a	10.03±3.22 a	6.73±4.07 b	46.13±12.98 a
strawberry without effective microorganism	10.57±1.84 a	63.64±14.47 a	67.16±20.88 a	10.72±2.64 a	4.26±1.27 a	52.19±17.68 a
harvest time I (8 of June)	8.93±0.96 a	58.25±6.55 a	63.99±9.89 a	11.24±0.66 b	5.43±0.57 b	47.33±8.75 a
harvest time II (15 of June)	9.88±1.67 a	41.39±9.14 a	65.31±27.59 a	9.55±3.80 a	3.58±0.87 a	52.19±22.51 a
harvest time III (22 of June)	12.24±1.02 b	82.64±9.37 b	65.78±22.71a	10.34±3.17 ab	7.47±4.82 c	47.97±14.23 a
p-value						
cultivation method	n.s.**	n.s.	n.s.	n.s.	<0.0001	n.s.
harvest time	0.0001	<0.0001	n.s.	0.0077	<0.0001	n.s.

*data are presented as the mean ± SD with ANOVA p-value; ** n.s.(statistically not significant); values labeled with the same letter are not significantly different (Tukay's honestly significant difference test, P=0.05)

Source: own work / Źródło: opracowanie własne

Fruits harvested in the third harvest time contained significantly more described phenolic acids. The effect of harvest time on the chlorogenic acid content in strawberry fruits was stated. Strawberries harvested in first harvest time contained significantly more chlorogenic acid and it was 8 mg/100 g FW (Fig. 1). The contents of caffeic and p-coumaric acids were significantly dependent on the applied effective microorganisms and harvest time. When applied to the cultivation of effective microorganisms, the fruits of the strawberry accumulated significantly more of both phenolic acids. In the case of the impact of the harvest time, increase in the production of caffeic acid in fruits in the second period was observed. For the p-coumaric acid content in fruits third harvest time was much better (Fig. 1). Only harvest time had a significant effect on the content of ellagic acid in the fruit. Strawberry collected in the third time was characterized by significantly higher content of this acid compared to fruit being collected in Ist and IInd harvest and it was properly 1,41 mg/100 g FW (Fig. 1).

In strawberry fruits six compounds belonging to the flavonoids group (quercetin-3-O-rutinoside, quercetin-3-O-glucoside, kaempferol-3-O-glucoside, luteolin, quercetin and kaempferol) have been identified. Only in the case of kaempferol-3-O-glucoside a significant effect of the absence of effective microorganisms on the content of this compound in fruit strawberries was found. Crops grown without the addition of effective microorganisms contained significantly more of this compound as well as in the case of the content of the kaempferol there was a significant effect of microorganisms on kempferol content in fruits strawberries. Fruit produced without EM contains similar value of kaempferol compared to fruit harvested from plants grown with the addition of EM (Fig. 2).

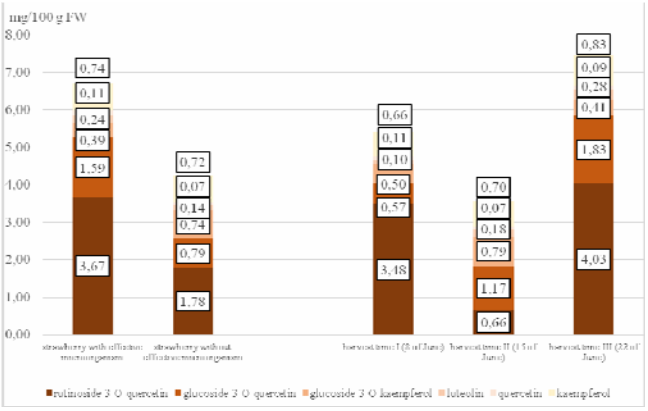

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Fig. 2. The content of identified flavonoids in experimental strawberry fruits depending on the cultivation method and harvest time

Rys. 2. Zawartość zidentyfikowanych flawonoidów w badanych owocach truskawki w zależności od metody uprawy i czasu zbioru

The harvest time of the strawberries had a significant impact on the content of flavonoids content in fruits of strawberries. In the third period of harvest the tested fruits of strawberry were characterized by considerably higher content of flavonoids (in spite of kaempferol-3-O-glucoside and quercetin).

In strawberry fruits three anthocyanins compounds have been identified (pelargonidine-3,5-di-O-glucoside, pelargonidine-3,5-di-O-rutinoside, cyanidine-3,5-di-O-glucoside). There was no impact of the application of effective microorganisms on the content of anthocyanins in strawberries (Fig. 3). Only the harvest time had a significant impact on the content of anthocyanins in fruits of strawberries, just one anthocyanin: pelargonidin-3,5-di-O-rutinoside. Strawberry fruits collected in the third harvest time were characterized by significantly higher content of pigment in the fruit and it was 2.71 mg/100 g FW.

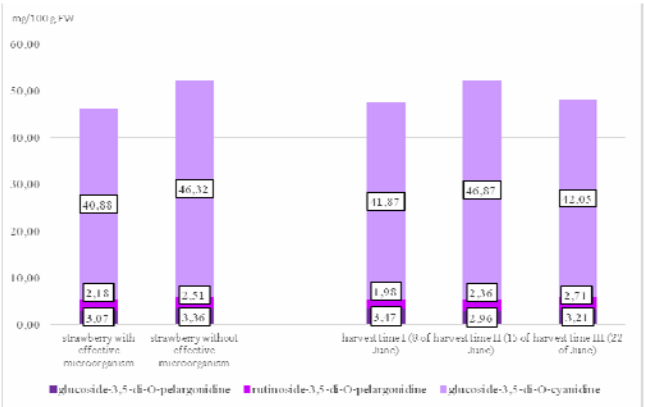

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Fig. 3. The content of identified anthocyanins in experimental strawberry fruits depending on the cultivation method and harvest time

Rys. 3. Zawartość zidentyfikowanych antocyjanów w badanych owocach truskawki w zależności od metody uprawy i czasu zbioru

4. Disscusion

The use of effective microorganisms in organic agriculture increases the plants vigor, enriches the soil with beneficial bacteria and fungi. It results in the better plant growth and better yields of high-quality fruit with biological value [7]. Total soluble sugars (TSS) is one of the most important features for strawberry quality determination. It is more than 90% of the dry weight of the fruit. Fruits with high content of sugar extract are sweeter and more accepted by the consumer [2]. The high content of total soluble sugars in strawberry fruits can be connected with photosynthetic plants activity and a higher sugars production [1]. In presented study, there was observed no effect of effective microorganisms on the dry matter content in the strawberry fruits. These results are different from those presented by Akhatou and Recamales [1], who claimed that the fruit harvested from plants grown with the use of effective microorganisms are characterized by a significantly higher sugar extract in fruits [1]. In this experiments, there was observed no effect of effective microorganisms on the vitamin C content. Different results were obtained by Hassan and Emam [5], who showed that the application of effective microorganisms increased the vitamin C content in strawberries by 2% in the first year and 1.7% in the second year of experiment [5]. Vitamin C is one of the most important factors creating the nutritial value of strawberries. A lot of published experiments focus on the effect of the content of vitamin C in fruits. The obtained results showed that the harvest time and temperature play important role for the accumulation of vitamin C in strawberries (tab. 1). Similar results were presented by Rahman [9], who showed that in

a lower temperature and moderate exposure on sunlights, but with long harvest time strawberry accumulates more vitamin C, compared to fruit cultivated with stronger sunlight and higher temperature [8]. Polyphenols have a strong anticancer and antioxidant properties that why these compounds are the subject of many studies. In the presented experiment there were neither observed significant influence of effective microorganisms on the total polyphenol content, nor the total phenolic acids in the strawberries. However, in the case of total flavonoids it was found significantly higher their concentration in fruits of plants grown with the use of effective microorganisms. Increasing the concentration of flavonoids in fruits and leaves of strawberries under the EM exposure is the result of increase in the plants resistance to pathogens or pests attack. This is the phenomenon of systemic acquired resistance. It involves the activation of the plants defense system in the health parts of the plants. The physiological mechanism of these phenomena consists in the production of special proteins associated with pathogenicity. The first signal to start response of plants is revealed by higher salicylic acid production and concentration in plant tissue. In the second step it leads to production and the accumulation of other phenols, and, in particular, the increase in the content of flavonoids and synthesis of many others proteins, as well as different secondary metabolites (fitoelaksyn). The chemical compounds belonging to this group may be affected by the toxic pathogens [6]. The effect of harvest time on the polyphenols content in the strawberry fruits is still understood. This paper is an attempt to extend the current state of knowledge about the contents of each fraction of polyphenols in strawberries depending on the harvest time. In the presented studies the harvest time was an important factor in determining the content of phenolic acids and flavonoids, but not total polyphenols and anthocyanins in strawberry fruits. Similar results were obtained by Lester at al. [8], who showed that fruits collected after each three days had different level of total flavonoids. No diversity in case of the total polyphenols and phenolic was observed. In the presented studies no significant effect of effective microorganisms on anthocyanin content in fruits strawberries was observed. Indeed there was a trend to higher content of these antioxidants in fruit from plants grown without EM. The same results were presented by Hassan and Emam [5], who achieved significantly lower contents of anthocyanins in fruit in combination with effective microorganisms compared to control, where EM was not used [5]. As is clear from the presented experiment the quality of strawberry fruits could be successfully modified by the use of appropriate fertilization and plant protection, as well as by the choice of proper harvest time.

5. Conclusions

1. The use of effective microorganisms in organic strawberries cultivation has affected significantly the content of total flavonoids and, in particular, quercetin-3-O-rutinoside, quercetin-3-O-glucoside, luteolin and quercetin as well as phenolic acids: caffeic and p-coumaric; Strawberry fruit collected from the plants grown without effective microorganisms were characterized by significantly higher content of gallic acid, kaempferol-3-O-glucoside compared to fruit cultivated with EM;
2. The fruits harvest time had important influence on the strawberry quality. Fruit harvested in third time were characterized by significantly higher content of dry matter content, vitamin C, flavonoids, quercetin and its derivatives, luteolin and kaempferol, gallic, p-coumaric and ellagic acids, compared to fruits collected in I and II time;
3. Strawberries are a very good source of numerous biologically active compounds. Their level could be successfully modified by the use of appropriate fertilization, plant protection, as well as by the choice of proper harvest time. Organic strawberries are recommended in diet as one of the best source of bioactive compounds with pro-healthy properties.

6. References

[1] Akhatou I., Recamales A.F.: Influence of cultivar and culture system on nutritional and organoleptic quality of strawberry J. Sci. Food Agric., 2014, 94, 866-875.
[2] Cordenunsi B.R., Genovese M.I., Nascimento J.R.O., Hassimotto N.M.A., dos Santos R.J., Lajolo F.M.: Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars Food Chem., 2005, 91, 113-121.
[3] EUROSTAT Data base, http://ec.europa.eu/eurostat.
[4] Giampieri F., Tulipani S., Alvarez-Suarez J.M., Quiles J.L., Mezzetti B., Battino M.: The strawberry: Composition, nutritional quality, and impact on human health. Nutrit., 2012, 28, 9-19.
[5] Hassan A.H., Emam M.S.: Improving fruit quality and storability of strawberry fruits by using pre and postharvest treatments. J. Am. Sci., 2015, 11, 44-60.
[6] Kowalska J., Sosnowska D., Remlein-Starosta D., Drożdżyński D., Wojciechowska R., Łopatka L.: Efektywne mikroorganizmy w rolnictwie ekologicznym. Instytut Ochrony Roślin - Państwowy Instytut Badawczy w Poznaniu, Mat. MRiRW z dnia 13.06. 2011, Nr PKre-029-6-3/11(150).
[7] Kumar N., Singh H.K., Kumar M.P.: Effect of organic manures and biofertilizers on growth, yield and quality of strawberry (*Fragaria x ananassa* Duch.) cv. Chandler. Trends Biosci., 2015, 8 (2), 490-492.
[8] Lester G.E., Lewers K.S., Medina M.B., Saftner R.A.: Comparative analysis of strawberry total phenolics via Fast Blue BB vs. Folin-Ciocalteu: Assay interference by ascorbic acid. Journal of Food Composition and Analysis, 2012, 27, 102-107.
[9] Rahman M.M.: Effect of planting time and genotypes growth, yield and quality of strawberry (*Fragaria × ananassa* Duch.). Sci. Hort., 2014, 167, 56-62.
[10] Singh R., Sharma R.R., Kumar S., Gupta R.K., Patil R.T.: Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (*Fragaria x ananassa* Duch.). Bioresour. Technol., 2008, 99, 8507-8511.
[11] Zasowska-Nowak A., Nowak P.: Prozdrowotne efekty konsumpcji truskawek. Przem. Ferment. Owoc. Warzyw., 2014, 7-8, 20-24.