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THE EVALUATION OF THE CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS IN THE OLD AND THE NEW PLUM CULTIVARS

Summary

The fruits of the old cultivars are valuable because of the taste and their culinary usefulness. The old cultivars of plants increase biodiversity in organic farms. Plum fruits are characterized by high healthy potential because they are rich in numerous biologically active compounds (polyphenols, especially phenolic acids, flavonoids and anthocyans), as well as vitamin C. In the presented work the plums of the old cultivars contained more vitamin C, phenolic acids and flavonols in comparison with new varieties of fruits. In the modern literature research on the comparison of the pro-healthy value of the new and the old plum cultivars is lacking. The experiment was carried out in 2017 using four varieties of the old and the new plums: Cacańska Rana, Opal, Ruth Gerstheter, Węgierka Wczesna (a gro up of the old cultivars) and Fryga, Kalipso, Katinka, Silvia (a group of the new cultivars). The content of dry matter (by scale method), vitamin C as well as polyphenols (by HPLC method) were determined in fruits. The obtained results were subject to statistical elaboration using Duncan's post-hoc test (α = 0.05). The obtained results indicate that plums of the old varieties contained slightly more vitamin C, total phenolic acids (including caffeic, ferulic, p-coumaric and chlorogenic acids) and flavonols compared to plums of the new cultivars. The Cacańska Rana cv. fruits were characterized by the highest content of identified and measured biologically active compounds in comparison with the fruits of the other tested plum cultivars.
***Key words**: plums, new cultivars, old cultivars, polyphenols, vitamin C, anthocyanins, flavonoids*

OCENA ZAWARTOŚCI ZWIĄZKÓW BIOLOGICZNIE CZYNNYCH W DAWNYCH I NOWYCH ODMIANACH ŚLIW

Streszczenie

Owoce dawnych odmian śliw są cenione ze względu na walory smakowe oraz ich przydatność kulinarną. Wzbogacają bioróżnorodność w gospodarstwach ekologicznych. Śliwki charakteryzują się wysokim potencjałem zdrowotnym, ponieważ są zasobne w liczne związki biologicznie czynne (polifenole, szczególnie kwasy fenolowe, flawonoidy i antocyjany), jak również witaminę C. W prezentowanej pracy owoce dawnych odmian śliw zawierały więcej witaminy C, kwasów fenolowych oraz flawonoli w porównaniu z owocami odmian nowych. W najnowszej literaturze brakuje badań dotyczących porównania wartości pro-zdrowotnej nowych i dawnych odmian owoców śliw. Doświadczenie przeprowadzono w roku 2017 z użyciem czterech odmian śliw dawnych i nowych: Cacańska Rana, Opal, Ruth Gerstheter, Węgierka Wczesna (grupa dawnych odmian) oraz Fryga, Kalipso, Katinka, Silvia (grupa nowych odmian). W owocach oznaczono zawartość suchej masy (metodą wagową), witaminy C, jak też polifenoli (metoda HPLC). Otrzymane wyniki poddano analizie statystycznej z zastosowaniem testu post-hoc Duncana (α=0,05). Otrzymane wyniki wskazują, że śliwki dawnych odmian zawierały nieznacznie więcej witaminy C, kwasów fenolowych ogółem (w tym kawowego, ferulowego, p-kumarynowego i chlorogenowego) oraz flawonoli w porównaniu z śliwkami odmian nowych. Owoce odmiany Cacańska Rana charakteryzowały się najwyższą zawartością zidentyfikowanych i zmierzonych związków biologicznie czynnych w porównaniu z owocami pozostałych badanych odmian śliw.
***Słowa kluczowe**: śliwki, nowe odmiany, dawne odmiany, polifenole, witamina C, antocyjany, flawonoidy*

1. Introduction

Plums are fruits with high pro-health potential. In the last time they are an object of interest in the nutritional and dietary field. Plum fruits contained numerous biologically active compounds, such as polyphenols, including phenolic acids, flavonoids and anthocyanins, as well as vitamin C [5]. Consumption of fruit, and in particular plums has a multidirectional, positive effect on the human body. A diet full of fruits helps to maintain a slim figure, and also counteracts the occurrence of many dangerous diseases referred to as non-infectious chronic diseases and osteoporosis [7, 8, 18, 22, 23]. Plums are a very good source of polyphenolic compounds in our diet. It is a very large group of compounds with a strong antioxidant effect [10]. The content of polyphenolic compounds, especially anthocyanins, depends on the color of the fruit and the cultivar. Due to the color and shape of plum fruit, we can divide them into round fruits with yellow skin and flesh (plums from the renkloda/greenge/ group). Fruits of this type will be rich in flavonoid compounds and xanthophylls and chlorophylls conditioning the formation of yellow color. The second group of plums is characterized by elongated fruits with purple color of the skin and yellow flesh (plums from the węgierka group). Fruits of this type are rich in anthocyanins in the skin, as well as flavonoids and xanthophylls in the fruit flesh. The third group of fruits has round fruits with yellow or pink color of the skin and the same flesh (mirabelka/mirabelle plum group). The fruits of these varieties are rich in phenolic acids, flavonoids, anthocyanins and xanthophylls in the skin as well as in the flesh [24, 27]. Plum fruits also contain pectin and fiber as well as valuable organic acids. The whole health potential of these fruits makes them a very good source of bioactive compounds in our diet. Due to the maintenance of high biodiversity in organic farming, it is recommended to introduce the old and the local cultivars of fruits as well as animal races. The main problem in plum cultivation relates to the susceptibility to fungal diseases. In accordance with legal requirements, organic farming aims to produce fruit with high nutritional value without using agricultural chemistry. Trees in the orchard are fertilized before planting with the compost or manure, and the plant protection consists mainly in enriching species biodiversity and prevention. The organic orchard uses a much larger spacing of trees, and most agrotechnical operations are performed manually. Soil in organic orchard is covered with graves and herbs, in contrast to conventional orchards with herbicide rows [15, 16]. Protection of trees against pests involves the use of natural enemies, both insects and birds. The breeding sites are improving and organizing, so that insectivorous birds nest in the ecological court. Pheromone traps and yellow sticky boards are a common protection method [17]. In the case of protection against fungal diseases, varieties with low susceptibility to these diseases are selected. In the old cultivars of plum there are those that have low sensitivity to fungal diseases [21]. Therefore, we want to find cultivars that will combine high biological value of fruits and low susceptibility to diseases. Many researches indicate that organic fruits (apples, pears, peaches, strawberries) are characterized by a higher content of dry matter, vitamin C and polyphenolic compounds, including flavonoids and phenolic acids [3, 20, 25]. In the modern literature there are no studies on the quality of fruit of various plum cultivars considering the origin and classification of cultivars as the old and the new group, with particular emphasis on the influence of the content of bioactive compounds in fruits.

2. Materials and Methods

The experiment was carried out in 2017 at the Organic Food Division, SGGW. Four cultivars of plums belonging to the old and the new cultivars were used for the experiment. These were respectively: Cacańska Rana, Opal, Ruth Gerstheter, Węgierka Wczesna (a group of the old cultivars) and Fryga, Kalipso, Katinka, Silvia (a group of the new cultivars). Both the old and the new cultivars were grow in plant collection ant only conventional way of cultivation were used. Fruits were obtained from the collection of fruits of the Institute of Horticulture in Skierniewice. 2 kg plums from each cultivars were pickled, ground and then freeze-dried using a Labconco Freezedrier 2.5 at a temperature of -40°C and a pressure of 0.100 mBa. After freeze drying, the samples were ground in a Mill A-11 laboratory mill. The milled plant material was poured into scintillation vessels and kept at -80°C to prevent the loss of biologically active compounds. The results of chemical analyzes were obtained in an dry matter, then the results were calculated into fresh matter and given in milligrams per 100 g FW. The content of dry matter (before freeze-drying process) in the fruit was determined by scale method [13], polyphenols with separation by HPLC [11], vitamin C by HPLC. Description: 100 mg of freeze-dried was extracted with 5% meta-phosphorus. The sample was mixed on vortex and then incubated in an ultrasonic bath (15 min, 20°C). The sample was then centrifuged (6000 rpm, 10 min, 0°C). The supernatant was taken from the test tube (without stirring) and transferred into the HPLC vials. The following analysis parameters were used: mobile phase acetic buffer (pH 4.4), Phenomenex Hydro 80-A RP column (250 x 4.6 mm), analysis time 18 min, detection 255-260 nm; L-ASC and DHA standards to identify Fluka and Sigma-Aldrich with 99% purity. Four replicates were made for each analytical combination. Five injections of L-ASC and DHA standards were prepared from standard solutions, standard curves for the tested components of vitamin C were determined. The chromatogram was read and individual compounds were identified based on the retention time of the standards.

3. Results

The data presented in the Table 1 indicate that only in the case of the total polyphenol content and total flavonoids the groups of plum cultivars differed significantly among themselves. In the case of total polyphenols (p=0,013), significantly more of these compounds were found in the new cultivars, while in the case of total flavonoids (p=0,0145) it was a group of the old cultivars. The remaining qualitative indicators examined did not differ significantly between plum groups.

In the group of the old cultivars, significantly more ($p<0.001$) dry matter was found for Wegierka Wczesna cv., while in the group of the new cultivars it was the Silvia cv. The content of vitamin C was significantly higher ($p<0.0001$) in the fruits of Silvia (the new cultivars) and Cacańska Rana (the old cultivars). An extremely high total polyphenol ($p<0.0001$) content was found in plums of Kalipso cv. (Table 1), it was a variety belonging to the group of the new cultivars, while in the old cultivars group it was Cacańska Rana. Significantly more total phenolic acids ($p<0.0001$) were found in the fruit Opal cv. and Silvia cv. Significantly more total flavonoids ($p<0.0001$), including anthocyanins ($p<0.0001$), were found in the fruit of Kalipso cv. (the new cultivar) and the Cacańska Rana cultivar belonging to the old cultivars group. Cacańska Rana was the cultivar significantly more rich in total flavonols, and among the new cultivars it was a Fryga.cv.

In the presented studies, the dominant vitamin C fraction in plum fruit is L-ascorbic acid. The second fraction (DHA acid) consitutes only about 1% of the total vitamin C. In the group of the old cultivars, significantly more ($p<0.001$) DHA was found in the fruits of Węgierka Wczesna, while in the group of the new cultivars it was Katinka. In the case of l-ascorbic acid, significantly more ($p<0.0001$) of this compound was found in the Cacańska Rana fruit and in the group of the new cultivars it was the fruit Silvia cv. (Fig. 1).

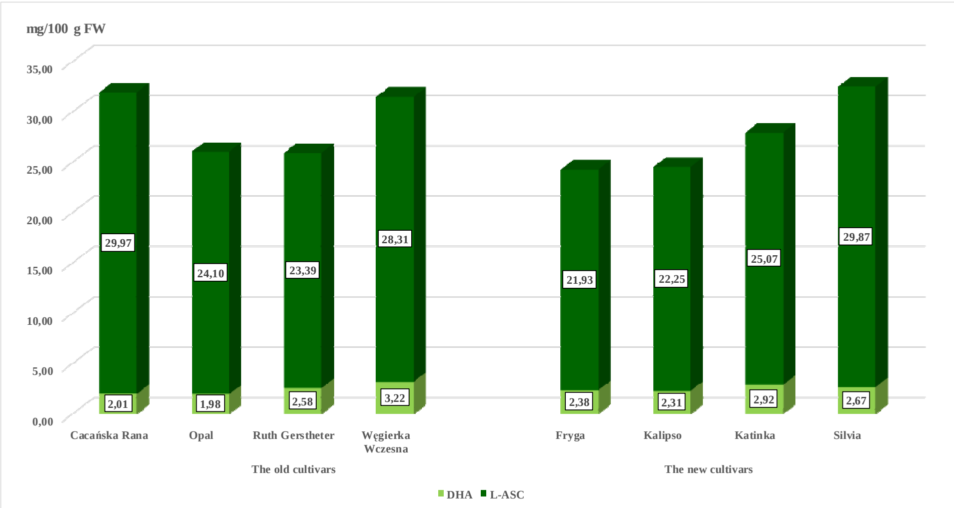
In the case of gallic acid content, significantly more ($p<0.0001$) of this compound was found in the Cacańska Rana cv, while in the group of the new cultivars it was the Katinka cv. Much more ($p<0.0001$) chlorogenic and ferulic acid was found in the fruits of the Węgierka Wczesna cv. while caffeic, p-coumaric and ferulic acid were found as dominant in the fruit of the Silvia cv. (the new cultivars). In the group of the old cultivars, we found considerably more ($p<0.0001$) caffeic and p-coumaric acids in the fruit of Opal cv. (Fig. 2).

Table 1. The content of dry matter (g/100 g FW), vitamin C and total phenolic compounds (mg/100 g FW) in examined plums from the old and the new cultivars. Mean value ± standard deviation; n=12

Tab. 1. Zawartość suchej masy (g/100 g ś.m.), witaminy C i związków polifenolowych (w mg/100 g ś.m.) w badanych śliwkach z grupy nowych i dawnych odmian. Wartość średnia ±odchylenie standardowe (liczba powtórzeń n=12)

		dry matter	vitamin C	total polyphenols	total phenolic acids	total flavonoids	total flavonols	total anthocyanins
The old cultivars	Cacańska Rana	13.52±0,14	31.98±0,38	41.00±0,83	4.47±0,19	36.53±0,70	3.08±0,10	33.45±0,80
	Opal	11.71±0,41	26.08±0,80	27.09±0,67	8.08±0,29	19.01±0,52	1.79±0,05	17.22±0,48
	Ruth Gerstheter	12.44±0,38	25.98±0,56	19.99±0,56	4.09±0,02	15.90±0,54	1.10±0,02	14.80±0,54
	Węgierka Wczesna	16.23±0,21	31.53±0,51	21.41±0,49	5.83±0,15	15.58±0,46	2.69±0,05	12.89±0,44
The new cultivars	Fryga	14.31±0,24	24.31±0,37	35.58±0,75	5.76±0,43	29.82±0,48	2.09±0,02	27.73±0,45
	Kalipso	12.82±0,29	24.56±0,54	85.58±3,10	3.70±0,19	81.88±2,92	1.40±0,03	80.48±2,91
	Katinka	13.16±0,72	27.99±1,33	35.80±0,70	4.22±0,20	31.57±0,59	1.53±0,14	30.04±0,68
	Silvia	14.68±0,35	32.54±0,81	30.52±0,54	6.88±0,27	23.64±0,73	1.81±0,05	21.84±0,74
mean value for group	the old cultivars	13.48±1,74	28.89±2,93	27.37±8,33	5.62±1,57	21.76±8,65	2.17±0,78	19.59±8,17
	the new cultivars	13.75±0,89	27.35±3,44	46.87±10,51	5.14±1,29	41.73±10,42	1.71±0,28	40.02±13,60
p-value								
group		n.s.	n.s.	0.013	n.s.	0.0145	n.s	0.0127
cultivars		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

n.s. - not significant difference *Source: own work / Źródło: opracowanie własne*



Source: own work / Źródło: opracowanie własne

Fig. 1. The content of vitamin C fraction in examined plum cultivars belonging to the old and the new cultivars groups p-value (DHA) <0.0001; p-value (L-ASC) <0.0001

Rys. 1. Zawartość frakcji witaminy C w badanych odmianach śliw należących do grupy dawnych i nowych odmian

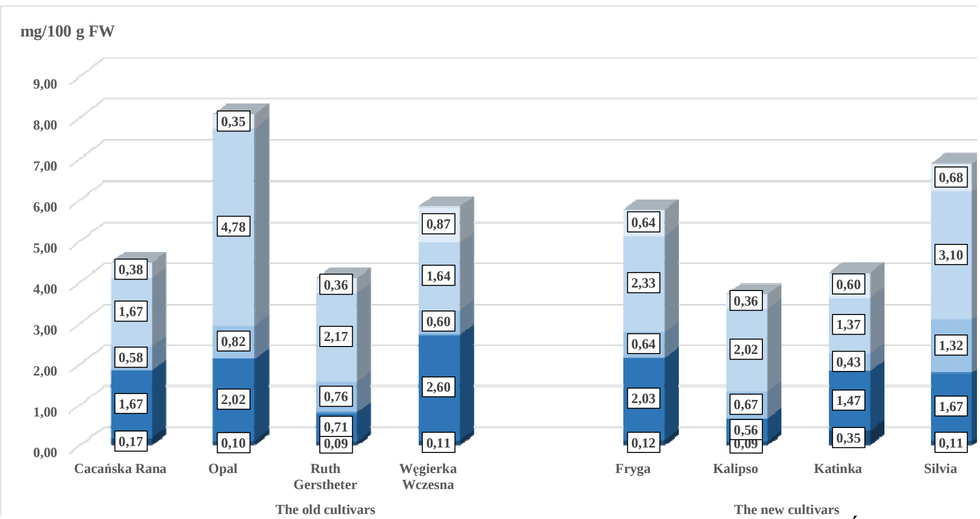


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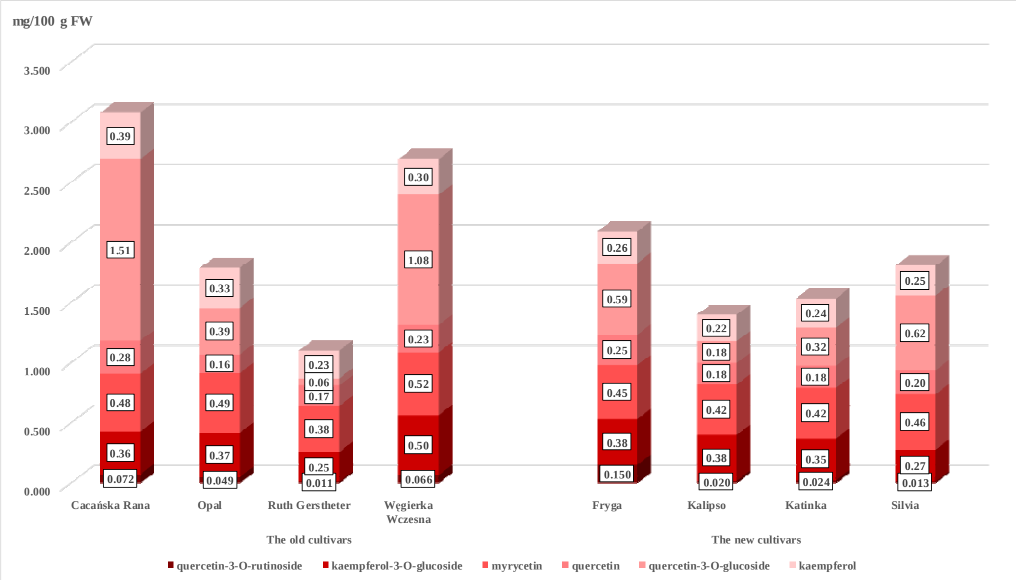
Fig. 2. The content of individual phenolic acids identified in examined plums cultivars belonging to the old and the new cultivars groups p-value (gallic) <0.0001; p-value (chlorogenic) <0.0001; p-value (caffeic) <0.0001; p-value (p-coumaric) <0.0001; p-value (ferulic) <0.0001

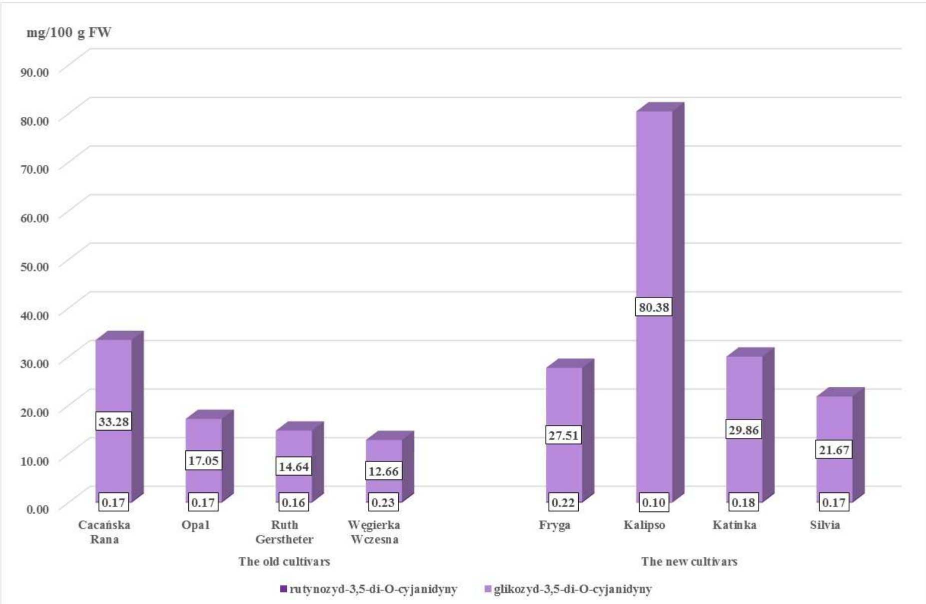
Rys. 2. Zawartość frakcji kwasów polifenolowych w badanych odmianach śliw należących do grupy dawnych i nowych odmian



Source: own work / Źródło: opracowanie własne

Fig. 3. The content of individual flavonols identified in examined plums cultivars belonging to the old and the new cultivars groups; p-value (Q3R) <0.0001; p-value (K3G) <0.0001; p-value (myrycetin) <0.0001; p-value (quercetin) <0.0001; p-value (Q3G) <0.0001; p-value (kaempferol) <0.0001

Rys. 3. Zawartość frakcji flawonoli w badanych odmianach śliw należących do grupy dawnych i nowych odmian



Source: own work / Źródło: opracowanie własne

Fig. 4. The content of individual anthocyanins identified in examined plums cultivars belonging to the old and the new cultivars groups; p-value (Cy-3,5-R) <0.0001; p-value (Cy-3,5-Gl) <0.0001

Rys. 4. Zawartość frakcji antocyjanów w badanych odmianach śliw należących do grupy dawnych i nowych odmian

In case of flavonol content, it was found that among the old cultivars, significantly more (p<0.0001) quercetin-3-O-rutinoside, quercetin, quercetin-3-O-glucoside as well as (kaempferol-3-O-glucoside and myrycetin) in the fruits of Węgierka Wczesna cv. A similar situation was with the fruit of the new cultivars. There was significantly more (p<0.0001)quercetin and kaempferol as well as their derivates in the fruits of Fryga cv. Only myrycetin and quercetin-3-O-glucoside have been found with considerably higher level in Silvia cv. (Fig. 3).

In the anthocyanin group, only two compounds: rutinoside-3,5-di-O-cyanidine and a 3,5-di-O-cyanidine glycoside were identified. 3,5-di-O-cyanidine and a 3,5-di-O-cyanidine glycoside were identified. The last one is the dominant antocyanin. In the case of rutinoside content, significantly more (p<0.0001) of this pigment was found in the fruits of the Węgierka Wczesna cv. (the old cultivars group) and in the fruit of the Fryga cv. (the new cultivars group). However, in the case of the cyanidin glycoside, significantly more (p<0.0001) of this pigment was found in the Cacańska Rana fruit and the Calypso fruits (Fig. 4).

4. Discussion

Plums are a very valuable source of biologically active compounds in our diet. The color of the fruit depends mainly on the distribution of colorants inside the fruit. In the plums' flesh and skin we can find compounds from the group of flavonoids and xanthophylls giving a yellow color and anthocyanins giving a purple coloration. Dry matter content is a very important fruit quality parameter. The content of dry matter depends on many physiological factors (state of maturity), agrotechnical (cultivation) or genetic (cultivar). In the presented experiment, plums from the group of the old cultivars were characterized by a lower content of dry matter (13.48 g / 100 g FW) in comparison with plums from the group of the new cultivars (13.75 g / 100 g FW), however differences were not statistically significant. In the presented experiment, plums of the old cultivars are more similar in quality to organic plums, while plums of the new cultivars come from a typical conventional orchard. The obtained results differed from those obtained by Lombardi-Boccia et al. [12], who showed no differences in the dry matter content between organic and conventional fruit (11.3 g / 100 g FW) and Daza et al. [6], who prowed that the organic fruit was characterized by significantly higher content of dry matter in fruit, respectively 14.0 g / 100 g FW compared to conventional fruits 12.49 g / 100 g FW. As mentioned, the dry matter content in fruit depends not only on the production system, but also on the variety.

In the presented experiment, the highest amount of dry matter was found in the fruits of Węgierka Wczesna (16.23 g /100 g FW), and the lowest level - in fruit of Opal cv. (11.71 g / 100 FW). Similar results were obtained by Wladkowiak-Tomaczak et al. [26], who obtained the highest dry matter content in plum fruit for the Węgierka Wczesna cv. (19.8 g / 100 g FW), and the lowest for the Węgierka Dąbrowicka cv. (12.6 g / 100 g FW). In the experiment presented by Daza et al. [6], it was shown that the range of dry matter content in organic plums was 8.8-15.1 g / 100 g FW while in the case of conventional plums it was 9.7-15.5 g / 100 g FW.

Plums are not so well source of vitamin C. The organic production system results in increased synthesis of vitamin C in crops [1, 11]. In the presented experiment plums of the old cultivars contained on average 28.89 mg / 100 g FW, and plums of the new cultivars 27.35 mg / 100 g FW (Table 1). Cultivar is the second important factor determining the content of vitamin C in plum fruit. The second important factor determining the content of vitamin C in plum fruit is a cultivar. In the presented experiment the highest amount of vitamin C have been found in the fruit of the Silvia cv., and the lowest level in the fruit of Fryga cv. (Table 1). Similarly reported Gil et al. [9]. They showed that the strongest factors determination of vitamin C content in plums it was cultivar both in the fruit flesh as well as in the skin.

Plum fruits contain numerous polyphenolic compounds. Most of them are in the skin - almost five times more than in the flesh, but also in both parts of the fruit there are other fractions of polyphenolic compounds. The content of these bioactive compounds in plum fruit is very variable and closes within the range of 38.1-672.0 mg / 100 g FW [5]. In the presented experiment, the total content of polyphenolic compounds amounted to 27.37 mg / 100 g FW for plum of the old cultivars, and for plums belonging to the new group of cultivars it was 46.89 mg / 100 g FW (Table 1). These results are more similar to those presented by Lombardi-Boccia et al. [12], who showed that there were 88 mg / 100 g FW in organic plums and 121,0 mg / 100 g FW in conventional plums. Significantly lower total polyphenol content in plum fruit presented by the Lombari-Boccia et al. [12] team results from varietal differences and fruit type, because yellow fruit was used in this experiment, so the polyphenol pool was due to the presence of only phenolic acids in fruit flavonoids.

In the presented experiment, the cultivars were represented by purple fruit plums (with anthocyanins contained in the skin, also included in polyphenolic compounds). Therefore, much lower values were obtained for plum trees of the old cultivars as well as new cultivars. The content of polyphenolic compounds in plums is strictly dependent on the cultivars - the most total polyphenols were found in the fruits of PR04-35 maple (383.0 mg / 100 g FW), and the least in Sun Breeze varieties (232.0 mg / 100 g FW).

In the presented experiment, the lowest level of total polyphenols were found in the fruit of the Ruth Gerstheter variety (19.99 mg / 100 g FW), and the most in the Kalipso variety (80.58 mg / 100 g FW). Chlorogenic acid is the predominant phenolic acid in plum fruit, but there are also cryptochlorogenic and neuro genogenic acids, and in smaller concentrations- caffeic and p-coumarin acid [26]. In the presented work plums of the old cultivars contained slightly more chlorogenic acid (1.75 mg / 100 g FW) in comparison with plums of the new cultivars (1.43 mg / 100 g FW) (Fig. 1). These results are significantly lower than those obtained by Lombardi-Boccia et al. [12], who obtained for organic plums the value of 37.5 mg / 100 g FW and 25.2 mg / 100 g. FW for conventional fruit. A similar situation occurred with the p-coumaric acid content. In the presented experiment, there was no difference in the content of this phenolic acid between the fruits of old and new varieties (Fig. 1). In the work of Lombardi-Boccia et al. [12] organic plums contained 8.5 mg / 100 g FW, and conventional plums 8,9 mg / 100 g FW p-coumaric acid. In the presented experiment, quercetin glycoside was the dominant flavonoid in the plum flesh and in the fruit of the old cultivars it was 0.76 mg / 100 g FW, while in the fruit of the new cultivars 0.43 mg / 100 g FW. The experiment presented by Lombardi-Boccia et al. [12] showed that the dominant flavonoid in plum fruit was pure quercetin and was in its organic fruit 30.2 mg / 100 g FW and in conventional fruit 19.2 mg / 100 g FW. However, it is important to remember about differences in the type of fruit in both experiments (yellow plums versus purple plums) and cultivars differences. The obtained results differed from those presented by Venter et al. [23], who also showed that among the eight derivatives of quercetin, rutinoside is the predominant compound, while quercetin in its pure form is not found in plums. The content of anthocyanins, which are responsible for the characteristic color of the plum fruit skin, is quite large (18-170 mg / 100 g FW). The content of these colorants in the peel depends on the location of the orchard (the conditions of sunlight and the presence of UV radiation). It is known that anthocyanins are produced intensively in response to strong UV radiation [4, 14, 19].

In the presented experiment, the total anthocyanins content for the old plum cultivars was 19.59 mg / 100 g FW, and for the new plum cultivars it was 40.02 mg / 100 g FW. The main purple colorants identified in the fruit skin were cyanidin and their derivatives (rutinoside and glucoside). In the presented experiment, this two anthocyanins were identified in the fruit. With the exception of the cyanidin glucoside, which occurred predominantly in the fruit of the new cultivars, the second anthocyanin was present only in trace amounts in the fruits of both compared groups and statistically these differences were not significant (Fig. 4). The content of cyanidine glucoside was the highest in the Kalipso cv (80.38 mg / 100 g FW), and the lowest in the fruit of the Węgierka Wczesna cv. (12.66 mg / 100 g FW). Venter et al. [23] reported similarly that the content of cyanidin in plum fruit depended mainly on the cultivar, followed by the state of maturity and the growing season. The most cyanidin glucosides were recorded in fully mature Ruby Red fruits (47.4 mg / 100 g. FW) and in the new PR04-19 clone (48.8 mg / 100 g. FW). The fruits harvested in the second year of the experiment contained significantly more anthocyanins compared to the fruits of the first year. It was closely related to the intensity of solar radiation, which was significantly higher in the second year of research.

5. Conclusions

1. The plums belonging to group of the new cultivars contained significantly more total anthocyanins as well as flavonoids and anthocyanins compared to the plums from the old cultivars group.
2. The plums from the old cultivars group contained not significantly more vitamin C, total phenolic acids as well as total flavonols compared to the new cultivars group.
3. In the group of the old cultivars Cacańska Rana is one of the best and in the new plum cultivars group there were two equal cultivars: Fryga and Silvia.
4. Despite differences between cultivars, it is worth pay attention to plums of the old cultivars, because they enrich our diet with numerous biologically active compounds from the group of polyphenols, therefore they are recommended for everyday consumption in order to promote health.

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