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The effect method of preservation on selected bioactive compounds and antioxidant activity in blackthorn fruits (*Prunus spinosa* L.)

Wpływ metody konserwacji owoców śliwy tarniny (*Prunus spinosa* L.) na zawartość wybranych substancji czynnych oraz aktywność przeciwutleniającą

Summary. The effect of freezing (M), freeze drying (L) and convection drying (S) of blackthorn fruits on the content of selected bioactive compounds and antioxidant properties compared to the control sample – fresh fruit (PK), was tested. Changes in the content of flavonoids, anthocyanins, pectins, general acidity and the ability to reduce the DPPPH radical, were determined. The obtained results showed that the freeze-dried fruits had the highest content of anthocyanins and flavonoids, respectively 0.07 and 0.17%. Pectin contents in freeze-dried and convectively dried fruits were similar at the level of 1.66 and 1.64% as well as for acidity respectively 0.076 and 0.071 $g \cdot 100\ g^{-1}$ infusion. Frozen fruits did not differ significantly compared to the control in terms of the content of anthocyanins, flavonoids, pectin and acidity. The highest scavenging capacity of free radical DPPH was characterized by the infusion obtained from fresh fruits.

Key words: *Prunus spinosa* L., blackthorn, preservation, anthocyanins, flavonoids, DPPH

INTRODUCTION

Blackthorn (*Prunus spinosa* L.) is a species of deciduous shrub belonging to the *Rosaceae* family. This plant is typical for temperate climate, it is common in Poland, often forms dense thickets at the bounds, forest edges and sunny slopes. Blackthorn produces branched stems up to 3 m high, the lateral shoots of which have been severely shortened [Strzelecka and Kowalski 2000, Senderski 2017]. Bushes begin flowering early, at the turn of April and May, becoming the spring benefit for bees [Buliński 2011].

Spherical, dark-blue fruits covered with a wax coating ripen at the turn of September and October, but remain on the bushes until late autumn. Their green flesh with a sour, tart flavor, adhered to the stone, becomes sweeter after becoming frozen [Kołodziej 2010, Senderski 2017].

Two medicinal raw materials are obtained from blackthorn: flowers and fruits. The flowers abundant in flavonoids are included in fruit teas and herbal mixtures with a cleansing, metabolism-regulating and diuretic effect. Fruits contain tannins, anthocyanins, flavonoids, organic acids and vitamin C [Strzelecka and Kowalski 2000, Senderski 2017]. They are used as a component of fruit teas [Adamczak et al. 2015] and jams, acetic marinades, compotes, jellies and alcoholic beverages. Among the latter are, e.g., “Pacharan” from Navarra in northern Spain, or “Tarninówka” from Tarnów [Ibarz et al. 1996, Karabela 2012].

In teas and mixtures containing blackthorn fruits (so-called *tarki*), they are usually present in the form of a dried product obtained by drying the raw material in a convection dryer heated to +60°C [Senderski 2017]. This process aims at reducing the water content, due to which the enzymatic reactions occurring in tissues and vital processes of microorganisms colonizing the dried material are inhibited. However, removal of water by evaporation does not fully protect the dried material from adverse physicochemical changes, such as oxidation, pectin crystallization or evaporation of odoriferous substances [Krzysztofik et al. 2015, Zalewska 2016].

Freeze drying (lyophilization) involves removing water from previously frozen product by ice sublimation. Typically, the process in which dehydration proceeds without the liquid state is carried out under reduced pressure in the range of 15–150 Pa. The advantage of lyophilization is the lack of thermal degradation of the material, as a result of which the thermo-labile and easily oxidizable compounds are protected. Moreover, the shape and appearance of the raw material is preserved. This method is characterized by high rate of rehydration of the product. On the other hand, the disadvantage of freeze-dried products is the reduction of volatile substances responsible for the scent and their high hygroscopicity and porosity, which favors oxidation. Countering this undesirable process is based on the use of hermetic packaging for lyophilizates. Nevertheless, in comparison with other drying methods, lyophilization guarantees a high-quality product [Misiak and Irzyniec 2009, Krzysztofik et al. 2015, Zalewska et al. 2016, Nowak and Nienautowska 2017].

Freezing is the most commonly used refrigeration process used to preserve fruits and vegetables. The process involves rapid cooling of the product, exceeding the cryoscopic point, until the temperature reaches –20 to –30°C inside the raw material and further storage at a temperature of minimum –18°C. Raw materials preserved using this method inhibit the action of microorganisms and slow down chemical reactions, as well as enzymatic and biological processes. It is the only method of food preservation that allows to preserve the characteristics of a raw material comparable to the fresh one referring to taste, fragrance, color. Properly conducted process allows the formation of small ice crystals and minor damage to the fruit structure. Freezing is the most advantageous method of preservation that allows to remain thermo-labile pro-health ingredients [Markowska and Polak 2014, Krzysztofik et al. 2015, Banaś and Korus 2016, Kwaśniewska-Karolak and Krala 2016]. It should be emphasized that despite the undoubted advantages of freezing, frozen food is not completely stable due to the changes taking place in the freezing concentration phase. During this process, enzymes and their substrates are regrouped in unfrozen water. This process leads to the aging of a product, despite the low temperature being maintained [Agoulon 2016, Wilczyński et al. 2018].

The purpose of the study was to determine the influence of selected preservation methods, such as freezing, convective and freeze drying on acidity, anthocyanins, flavonoids and pectin contents as well as antioxidant properties of blackthorn fruits.

MATERIAL AND METHODS

The subject of the study consisted of blackthorn fruits obtained from the natural state near Zamość. The raw material immediately after the purchase in October 2017 was washed and dried. Seeds were then removed and divided into three parts that were maintained. One of the samples was dried convectionally in a dryer (Binden type FD 53) at a temperature of +60°C until a constant mass. The other two lots of fruits were frozen at –20°C using a freezer without forced air circulation. Subsequently, one of the frozen samples was dried in a lyophilizer Alpha-1-2-LD plus (Christ) for 24 hours at a pressure of 0.054 mbar and an ice condensation temperature of –65°C. Plant material obtained during conservation was immediately subjected to physicochemical analysis to minimize the processes taking place due to the access of oxygen. In order to reduce differences in the dry matter content between frozen vs. dried and freeze-dried fruits, after determining the moisture content of the raw material at the level of nearly 80%, it was decided to increase the mass of frozen samples five times.

Pectin content was determined by Morris method modified by Pijanowski [1973] and achieved results were expressed in %. The assessment was based on the measurement of dry filters weight before and after pectin precipitation using acetone from previously prepared extract.

An infusion of test fruits was prepared to make assessment of acidity and antioxidant properties. For this purpose, 1 g of dried and lyophilized fruits and 5 g of frozen material was weighed on analytical balance RADWAG AS 220/C/2 with an accuracy of 0.0001 g. Fruits were immersed in 100 ml of hot distilled water at 90–100°C and covered with a pan. Extraction was carried out for 15 min.

Determination of acidity of infusion made of blackthorn fruits was made by titration method according to Polish Standard PN-EN 12147 [2000]. Total acidity was expressed in g 100 g^{-1} infusion re-calculated onto malic acid. On the other hand, analysis of antiradical properties of infusions was carried out using the methodology proposed by Sanchez-Moreno et al. [1998] modified by Sielicka and Samotyja [2013], which is based on the use of the DPPH radical. The radical (2,2-diphenyl-1-peryrylhydrazyl) is stable under normal conditions, while in the presence of an antioxidant, it is reduced, which is accompanied by a change in the color of the solution from violet to yellow and a decrease in absorbance at 515 nm. The antioxidant activity was expressed as the reduction in the DPPH radical expressed as a percentage after sample incubation within 30 minutes in a darkened place relative to the initial absorbance of the pure DPPH radical.

In turn, the anthocyanin content was determined based on the differential pH measurement method [Wrolstad 1993], the essence of which is the measurement of difference in absorbance at pH 1 and at pH 4.5. Anthocyanins at pH 1 occur in the form of red flavyl cation, while at pH 4.5 they are transformed into colorless pseudo-alkaline.

The amount of flavonoids was determined according to the methodology presented in the Polish Pharmacopoeia VI [2002] based on the formation of yellow flavonoid complexes with aluminum. Obtained results were given as re-calculated onto quercetin.

Data were statistically processed using the Statistica 12 software. The obtained results were given as means of 3 replicates. Achieved values were subjected to variance analysis (ANOVA) and difference significance was examined using the Tukey test at the significance level of $\alpha = 0.05$. Values marked on the charts with the same letters do not differ significantly ($p < 0.05$). In addition, Pearson's linear correlation coefficients were calculated to determine the dependence of selected parameters.

RESULTS AND DISCUSSION

Application of three different methods of blackthorn fruits preservation: freezing (M), freeze drying (L) and convection drying (S) contributed to obtaining a strong differentiation among analyzed parameters. Results obtained during individual analyses indicate higher content of anthocyanins, flavonoids, pectins and acidity in dried and lyophilized fruits. Reasons for this situation should be found primarily in better extractability of tested substances, occurring as a result of damage to the cell walls during heat treatment and freeze drying [Nowacka et al. 2010]. It should be emphasized that properly conducted freezing and freezing storage of food products allows to maintain the nutritional value close to that of fresh raw material [Kwaśniewska-Karolak and Krala 2016, Wilczyński et al. 2018].

Significantly higher content of anthocyanins and flavonoids was recorded in fruits subjected to lyophilization in relation to other samples (Figs. 1 and 2). Frozen and fresh fruits contained similar contents of both compounds. Higher content of flavonoids characterized samples subjected to freeze-drying (0.07%) and drying (0.05%) as compared to fresh (0.02%) and frozen (0.01%) fruits. Synowiec-Wojtarowicz et al. [2014] showed that thermal treatment positively influences the content of flavonoids in fruit juices – in the case of orange juice, they recorded the increased content by 31%. The authors indicate that the heating process leads to decomposition of cell walls or the occurrence of chemical reactions, as a result of which the content of antioxidants increases. Piasek [2010] reported that thermal treatment of chokeberry juice leads to an increase in quercetin content in the analyzed material – in fresh juice, the author did not detect this compound, but it appeared only after thermal treatment (the highest content of 0.027 mg of quercetin ml^{-1} recorded after 4 hours of heating at +100°C). The content of flavonoids obtained during the experiment in all samples was lower than that indicated by literature references that report the amount at the level of 0.12–0.23% [Leja et al. 2007, Pelc et al. 2010]. The reasons can be found in the biochemical variability of fruits depending on the region of origin, soil and climatic conditions. Pelc et al. [2010], after the analysis of plant material obtained from 2 separate buckthorn populations located in eastern Poland, pointed to the occurrence of genetic variation and differences in chemical composition – fruits from the Bieszczady were more abundant in flavonoids, while those from Podlasie – in phenolic acids.

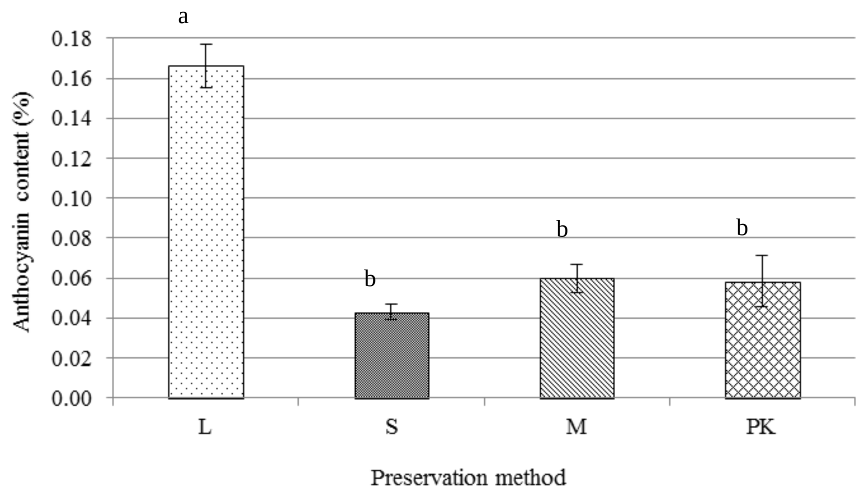

Fig. 1. Anthocyanins content in blackthorn fruits depending on preservation methods: L – freeze-drying, S – convection drying, M – freezing, PK – control sample

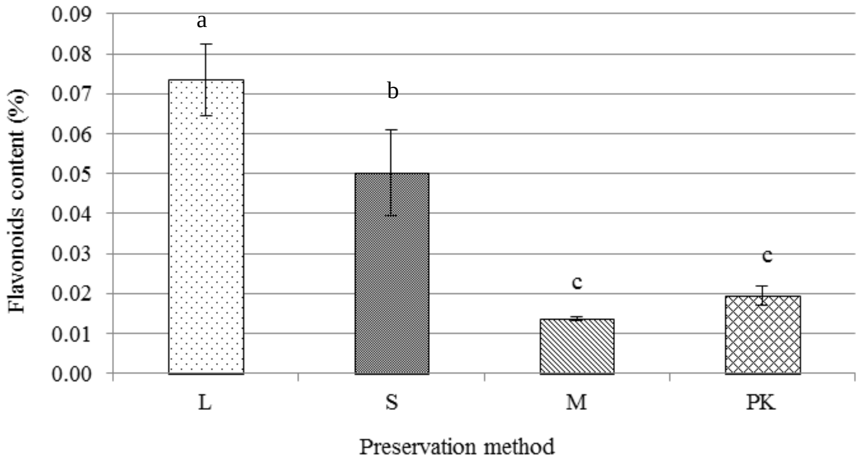

Fig. 2. Flavonoids content in blackthorn fruits depending on preservation methods expressed as equivalent of quercetin: L – freeze-drying, S – convection drying, M – freezing, PK – control sample

At the same time, negative effect of high temperature on the anthocyanin content in the analyzed samples was recorded. In convectively dried fruits, significant reduction in the share of this group of compounds has been demonstrated, but these differences were not statistically significant. In dried material, anthocyanins content was 4 times lower than in the case of lyophilizate – 0.04% and 0.17%, respectively. Ścibisz et al. [2010], when analyzing thermal degradation of anthocyanins in blueberry fruits, showed that heat treatment of fruits contributes to the breakdown of tested compounds. They also indicate other factors favoring decomposition of anthocyanins, which include, among others, pH and the presence of oxygen, sugars, metals, proteins or other polyphenolic compounds in the environment. Also Piątkowska et al. [2011] report changes in raw materials abundant in anthocyanins. They state that heating accelerates the oxidative polymerization processes of these colored compounds. Walkowiak-Tomczak et al. [2007], when examining the content of anthocyanins in the fruits of Polish plum varieties, obtained results at the level of 0.38–0.73%. Leja et al. [2007] indicate a 0.67% share of anthocyanins in fresh blackthorn fruits.

Gozdecka et al. [2015] indicate that pectins can stabilize anthocyanins and protect them from degradation. When analyzing the obtained results, there was a positive moderate correlation (R = 0.46) between the content of pectins and anthocyanins in tested samples.

Pectin content and acidity of studied raw material was significantly differentiated by the methods of maintenance (Figs. 3 and 4). In the freeze-dried and convectively dried fruits, similar percentage of pectins (1.66% and 1.64%) and acidity (0.076 and 0.071 g 100 g^{-1} of infusion) were recorded. Both parameters reached significantly lower values in the case of fresh and frozen fruits – 0.92 and 1.12%, 0.018 and 0.015 g 100 g^{-1} of infusion, respectively. Moreover, there was a strong correlation between pectin share and acidity of the fruits tested (R = 0.94). Literature references report lower pectin content oscillating around 0.73% [Sadowska et al. 2014]. Differences between obtained results and literature data may be due to the use of raw material with heterogeneous maturity. Unripe fruits are characterized by higher content of pectins, which along with the progressive maturation process, are degraded to monosaccharides [Nahorska et al. 2014]. When analyzing the total acidity of the plum fruits (*Prunus domestica* L.), Pobrzeżny and Wszelaczyńska [2013] presented results different from those obtained in the present experiment. They indicate that freezing and drying does not significantly increase the acidity of preserved fruits compared to the fresh ones.

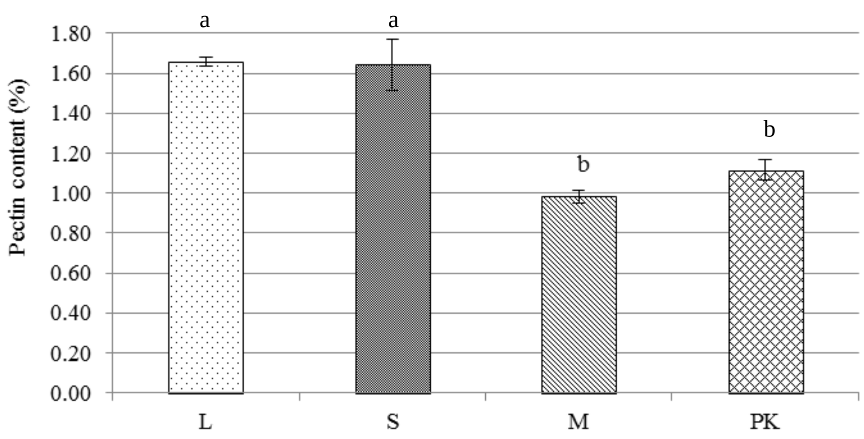

Fig. 3. Pectin content in blackthorn fruits depending on preservation methods: L – freeze-drying, S – convection drying, M – freezing, PK – control sample

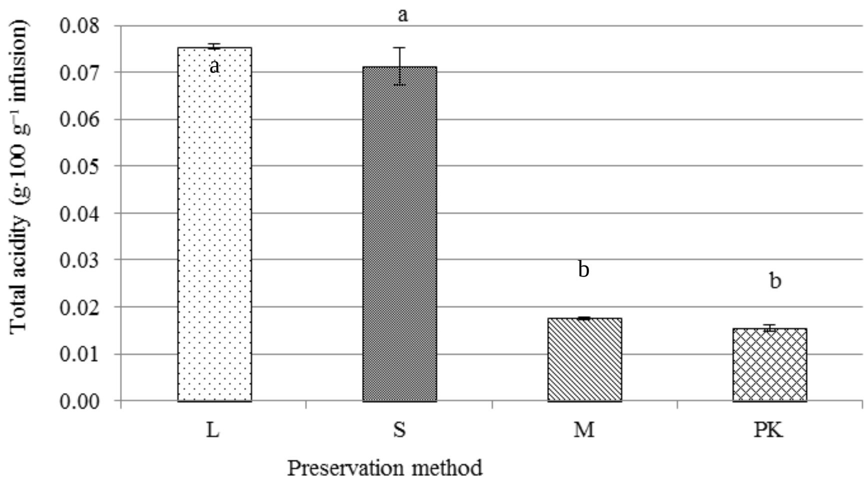

Fig. 4. Total acidity of blackthorn fruits depending on preservation methods: L – freeze-drying, S – convection drying, M – freezing, PK – control sample

The analyzed aqueous extracts from blackthorn fruits were characterized by diversified ability to scavenge free radicals. The highest concentration of DPPH was neutralized by infusions made from dried fruits (87.4%) and frozen fruits (85.2%), whereas extract from fresh blackthorn fruit scavenged the DPPH radical the weakest (Fig. 5). Kamińska and Kołton [2007] evaluated the ability of DPPH reduction by blackthorn fruit extracts stored at –20°C. The authors state that the investigated fruits showed reduced antiradical activity in comparison to fruits of hawthorn, dogwood, wild rose and elderberry (81.08–95.54% reduced radical) – while blackthorn after 30 minutes neutralized 55.35% of the free radical introduced.

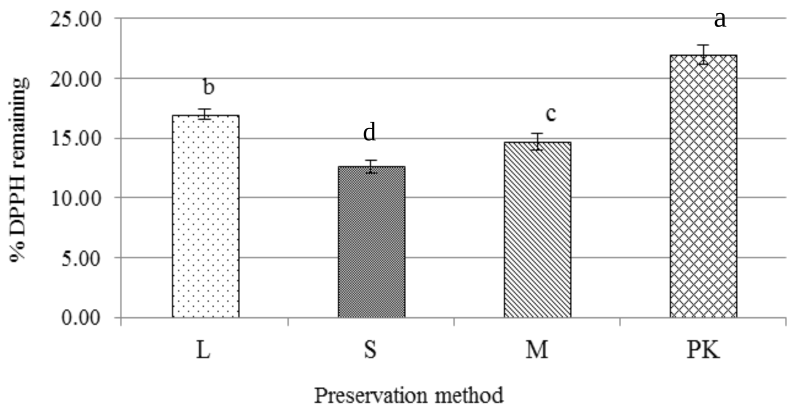

Fig. 5. Antioxidant activity of blackthorn fruits depending on preservation methods: L – freeze-drying, S – convection drying, M – freezing, PK – control sample

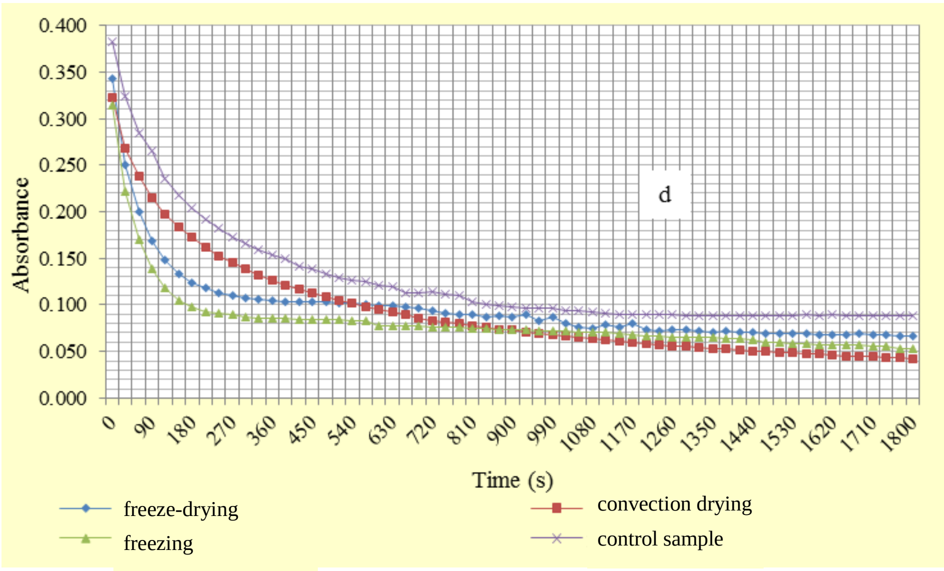

Fig. 6. Scavenging capacity of the DPPH free radical by aqueous extracts of blackthorn fruits depending on preservation methods

Method of blackthorn fruits preservation differentiated the course of DPPH radical binding by antioxidant system present in the analyzed aqueous extracts. The greatest ability to neutralize the radical in the first 5 minutes of the reaction characterized the extract obtained from frozen and lyophilized fruits. In turn, the reaction balance was achieved the fastest (about 1020 s) by fresh fruit extract. Based on the reaction kinetic curve, it was estimated that at 1800 s, in the case of a dried fruit sample, the largest amount of DPPH radical was scavenged, but the reaction duration was not sufficient to stabilize the absorbance.

CONCLUSIONS

1. Tested methods of blackthorn fruits preservation had significant influence on the content of examined substances (anthocyanins, flavonoids, pectins) and acidity.

2. Higher values for all the analyzed parameters were recorded in fruits subjected to the processes of freeze drying and drying.

3. Comparing selected methods of preservation, it was assessed that due to similar phytochemical properties of the raw material, freezing preserves properties similar to fresh fruit.

4. Based on the obtained results, it is estimated that the most preferred method of preserving the blackthorn fruits is freeze-drying, because it allows obtaining material with the highest concentration of active compounds in comparison to convective drying and freezing.

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Streszczenie. W pracy dokonano oceny wpływu mrożenia (M), liofilizowania (L) i suszenia konwekcyjnego (S) owoców tarniny na zawartość w nich wybranych substancji aktywnych oraz właściwości przeciwutleniające. Określono zmiany zawartości flawonoidów, antocyjanów, pektyn, kwasowości ogólnej oraz zdolność do redukcji rodnika DPPH. Na podstawie otrzymanych wyników stwierdzono, że w owocach suszonych metodą liofilizacji odnotowano największą zawartość antocyjanów i flawonoidów, odpowiednio 0,07 oraz 0,17%. W owocach liofilizowanych oraz suszonych konwekcyjnie zarówno ilości pektyn były zbliżone – na poziomie 1,66 oraz 1,64%, jak i kwasowości – odpowiednio 0,076 oraz 0,071 $g \cdot 100\ g^{-1}$ naparu. Owoce mrożone nie różniły się istotnie statystycznie w odniesieniu do próby kontrolnej w przypadku zawartości antocyjanów, flawonoidów, pektyn i kwasowości. Największą zdolnością zmiatania wolnego rodnika DPPH charakteryzował się napar otrzymany ze świeżych owoców.

Słowa kluczowe: *Prunus spinosa* L., tarnina, konserwacja, antocyjany, flawonoidy, DPPH

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