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The influence of selected preservation methods and storage time on the content of antioxidants in blackthorn (*Prunus spinosa* L.) fruits

Wpływ wybranych metod utrwalania oraz czasu przechowywania na zawartość przeciwutleniaczy w owocach tarniny (*Prunus spinosa* L.)

Summary. The aim of the study was to determine the influence of selected methods of preservation and storage time on the content of antioxidants in blackthorn fruit (*Prunus spinosa* L.). Preserved fruits were stored for 13 and 26 weeks without light at room temperature (freeze-dried and convection dried) and at –20°C (frozen samples). It was shown that both the method of preservation and the storage time had significant influence on the content of polyphenols (anthocyanins, flavonoids, total polyphenols) and antioxidant properties (Fe^{3+} reduction strength) of blackthorn fruit. As the storage time progressed, the anthocyanins content decreased in all preservation variants. In the case of frozen fruit after 26 weeks of storage, the content of flavonoids (by 12.5%), total polyphenols (by 48%) and the ability to reduce the iron ions were increased (by 55%). In turn, in the dried fruits after this period, the anthocyanins content, the polyphenol content and reduction strength were reduced. The highest values of the parameters evaluated after the assumed storage time has been demonstrated in lyophilizates.

Key words: *Prunus spinosa* L., anthocyanins, flavonoids, total polyphenols, antioxidant activity, preservation, storage

INTRODUCTION

Blackthorn (*Prunus spinosa* L.), belonging to the *Rosaceae* family, occurs in the form of thorny bushes or, more rarely, small trees. It often forms dense thickets on sunny slopes, bounds and railway embankments. This wild, occurring in Europe, species of plum gave birth to the common prune (*Prunus domestica* L.) as a result of spontaneous crossing with cherry plum (*Prunus cerasifera* L.) [Michalska and Łysiak 2014].

Blossoms (*Pruni spinae flos*) and fruits (*Pruni spinosae fructus*) are medicinal materials the most frequently obtained from blackthorn. In addition, folk medicine recommends the leaves of this plant (*Folium Pruni spinosae*) as a "blood cleaning agent" whereas root bark (*Cortex Pruni spinosae*) is recommended in the case of fever [Hoppe 1975, Palczewska and Owczarek 2002]. Flowers, that are an abundant source of flavonoids (on average over 1.5%), among which the largest share is isoramnetin 3-glucoside, kaempferol 3-O-glucoside, (+)-catechin and (-)-epicatechin gallate, serve for preparing the infusions with diuretic, metabolism-stimulating and elasticizing the blood vessels properties [Węglarz 2010, Senderski 2017].

On the other hand, spherical, dark navy, covered with wax coating, fruits are a raw material for the production of agents with astringent, anti-inflammatory, antiseptic and constipation features [Senderski 2017]. They are also used to make fruit teas [Adamczak et al. 2015], jams, acetic marinades, compotes, jellies and alcoholic beverages (e.g. "Pacharan" from Navarra in northern Spain, or "Tarninówka" from Tarnów) [Ibarz et al. 1996, Karabela 2012]. The blackthorn fruits are abundant in polyphenols, including tannins (about 0.5%), anthocyanins (0.06%), flavonoids (0.02%), phytosterols, organic acids and vitamin C (over 18 mg · 100 g DM^{-1}). Among the polyphenolic compounds, significant content of chlorogenic acid has been recorded (above 168 mg · 100 g DM^{-1}). In contrast, the prevailing sterol in fruit is β-sitosterol, the average content of which is 91.45 mg · 100 g DM^{-1} [Węglarz 2010, Olesińska et al. 2018].

Due to the periodicity of blackthorn fruit occurrence, an important issue is its preservation and storage under conditions that make it possible to preserve the pro-health and organoleptic properties of the raw material. One of the methods that allows to preserve the natural taste, smell and color of fruit is freezing [Kwaśniewska-Karolak and Krala 2016]. Properly conducted freezing process also contributes to the preservation of nutritional value and chemical composition in frozen food (especially thermo-labile components) at the level similar to that in fresh fruit [Banaś and Korus 2016, Wilczyński et al. 2018]. Another method of preservation is freeze drying, i.e. lyophilization. Lyophilized raw materials are characterized by good quality and durability, mainly due to inhibition of the majority of chemical and microbiological reactions and reduction of oxidation during drying [Sadowska et al. 2017]. Lyophilizates, however, are characterized by high porosity and hygroscopicity, resulting from the presence of amorphous structures. Both parameters favor rehydration and oxidation, which leads to the loss of valuable bioactive components. These processes can be limited by coating the lyophilizates with biopolymers or using hermetic packaging during the storage [Nowak and Nienautowska 2017]. However, the most popular method used in the preservation of plant material is convective drying. This method, unlike the previous two, is based on the process of removing water using warm air, which can affect the quality of the product obtained. In addition, high temperature can negatively affect the physicochemical (degradation of vitamins, shape changes, hardening of the material) and organoleptic properties, including color (thermal distribution of pigments), taste and smell (volatilization of essential oils) dried raw material [Czajkowska and Kowalska 2017].

The aim of the work was to assess the effect of the preservation method (freezing, freeze-drying, convective drying) and storage time on the content of polyphenols (anthocyanins, flavonoids, total polyphenols) and antioxidant properties (strength to Fe^{3+} ions reduction) of blackthorn fruit. On this basis, an attempt was made to indicate the method of preserving the blackthorn fruit, which allows preservation of the beneficial values of the raw material.

MATERIALS AND METHODS

The blackthorn fruit was obtained in 2017 from its natural state (from around Zamość, Poland). First, the fresh blackthorn raw material was characterized by randomly selecting 400 fruits, that were washed and dried, and their mass and diameter were then evaluated. In the subsequent stage, the stones were removed and weighed, which made it possible to assess their share in the blackthorn fruit. Obtained pulp was divided into three batches, which were subjected to preservation. The first one was subjected to convection drying at +60°C (Binden dryer type FD 53) until a constant mass was obtained. The other two lots of fruit were frozen at –20°C. One of them was freeze-dried in a lyophilizer (Christ Alpha-1-2-LD plus) for 24 hours at a pressure of 0.054 mbar at an ice condensation temperature of –65°C. After the preservation process, each batch was divided into three other parts. Samples subjected to drying and lyophilization were milled. One of them was immediately analyzed, while the other two were packed in polypropylene bags and stored for 13 and 26 weeks without light at room temperature (freeze-dried (L) and convective dried (S) and at –20°C (frozen samples).

In the preserved plant material, the anthocyanin content in reference to cyanidin-3--O-glucoside (Cy3G) was determined using the differential pH measurement method [Wrolstad 1993], flavonoids – based on quercetin, according to the Polish Pharmacopoeia VI [2002] and total polyphenols – using the Folin-Ciocalteu reagent, calculated as caffeic acid (CAE) [Singleton and Rossi 1965]. In addition, the reduction strength was measured using the FRAP method [Benzie and Strain 1996]. The obtained data was expressed in terms of mmol of reduced $Fe^{3+} \cdot$ g fruit^{-1}. Results were given as means of 3 replicates. In order to limit differences in the dry matter content between frozen, dried and lyophilized fruit, 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.

Results concerning the content of anthocyanins, flavonoids, total polyphenols and antioxidant properties (Fe^{3+} ion reduction strength) were subjected to a two-factor analysis of variance and the smallest significant differences were calculated with the Tukey test at the 0.05 confidence level. Numerical data was statistically developed using the Statistica 6.0 software.

RESULTS

Blackthorn produces relatively small fruit with a greenish flesh hardly peeling from the stone. Its dimension was assessed by measuring the length and width. The average value of both parameters in the tested sample was 13.9 and 12.6 mm, on average. In turn, the average weight of 100 fruits amounted to 166.0 g, the share of stones in the total weight of fruit – 27.3%, and the content of dry matter in the pulp – 21.9% (Table 1).

Blackthorn fruits intended for consumption should be characterized, among others, by large size and good health. In contrast, parameters desired by the industry, in addition to the size of fruit, also include: uniform shape, ease of moving the pulp away from the stone and yellow color of the pulp. Furthermore, an important criterion is relatively high content of dry matter, which in the fruit of blackthorn is in the range of 10–30%, with average value of 16% [Michalska and Łysiak 2014]. However, usually a small mass of individual fruits, difficulties in separating the flesh and a bitter taste are the main reason for the rare use of blackthorn in the processing industry.

Table 1. Characteristics of fresh blackthorn fruit

Analyzed feature	Mean	Standard deviation
Average weight of 100 fruits (g)	166.0	0.333
Average weight of 100 stones (g)	45.0	0.110
Share of stones in fruits (%)	27.3	3.633
Number of fruits (pcs. · 100 g^{-1})	69.3	0.829
Fruit length (mm)	13.9	1.50
Fruit width (mm)	12.6	1.13
Fruit length to width ratio	1.10	0.077
Stone length (mm)	9.48	0.858
Stone width (mm)	5.46	0.390
Stone length to width ratio	1.74	0.080
Content of dry matter in pulp (%)	21.9	0.258
Total polyphenol content (mg CAE · g $fruit^{-1}$)	4.09	0.176
Reduction strength (mmol Fe^{3+} g $fruit^{-1}$)	0.14	0.001

Compared methods of preservation significantly affected the anthocyanin content in blackthorn fruits. Particularly positive effects were recorded as a result of freeze-drying, which contributed to a significant increase in the concentration of colored compounds in the assessed samples. It seems that this can be explained by the increase in the dry matter content in the fruit pulp as a result of its dehydration. A similar effect in freeze-drying of fruit was obtained by: Wojdyła et al. [2007] as well as Pobrzeżny and Wszelaczyńska [2013]. However, this statement may only be adequate when comparing a freeze-dried sample with a frozen one, because in the case of dried material, the dry matter content in the blackthorn fruit also increased, but this did not translate into an increase in the anthocyanin content. The reasons for this situation should be seen in thermal degradation of the described compounds, which is confirmed by the research results reported by Ścibisz et al. [2010] and Sikorski [2002], who indicate that oxidative polymerization of anthocyanins is intensified during thermal fruit preservation. In turn, Piątkowska et al. [2011] reported that irreversible transformation of anthocyanin pigments, resulting from this process, can be observed in long-stored products, which is determined by the change in the fruit color from red to red-brown.

The content of anthocyanins during storage (26 weeks) underwent significant changes (Fig. 1). In the case of samples subjected to lyophilization and drying, a decrease in the concentration of the analyzed compounds was recorded along with the duration of their storage period. After 26 weeks of storage, the anthocyanins content was reduced by 26.7% and 48.5%, respectively, compared to the control sample. However, more complex changes occurred in frozen samples. After 13 weeks, the anthocyanins content decreased by 39.16% compared to the control sample (non-stored). In turn, after 26 weeks, an increase in the concentration of anthocyanins (by 27.62%) was demonstrated in relation to samples stored for a period of 13 weeks.

Tryzno et al. [2015] indicated that the storage time at +25 ºC did not significantly affect the content of anthocyanins in lyophilized raspberry fruits for the first 8 weeks. However, a significant decrease was recorded after 24 and 39 weeks (by 17% and 25%, respectively). Also Syamaladevi et al. [2011] recorded a decrease in the content of anthocyanins in lyophilized raspberries (by 27–32%) after 22 and 39 weeks of storage, respectively. However, in frozen fruits stored at –20 ºC, the above authors found the increased anthocyanins concentration by 38% after 22 weeks and 12% after 39 weeks of storage.

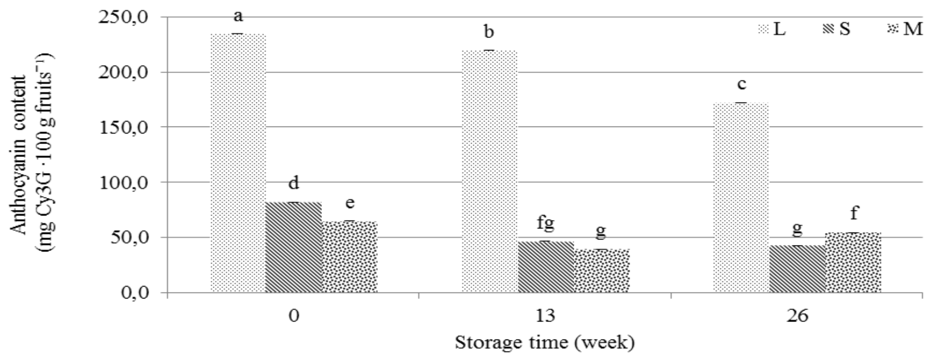

Fig. 1. Effect of preservation method and storage time on the anthocyanin content in blackthorn fruits. L – freeze-drying, S – convection drying, M – freezing, Cy3G – cyanidin-3-O-glucoside. Means followed by the same letter do not differ significantly at p = 0.05

The flavonoid content was significantly differentiated by the method of preservation and storage time (Fig. 2). The highest content of flavonoid compounds was demonstrated in samples subjected to lyophilization and drying, which were stored for a period of 13 weeks. In turn, after the next 13 weeks of their storage, a significant decrease in the content of this group of compounds was found: in lyophilizates – by 48.84%, and in dried material – by 35.14%. Such situation might be the result of a greater aeration of powdered blackthorn fruit, which contributes to the oxidation of flavonoids. The described process, to a limited degree, concerns the frozen product [Szajdek and Borowska 2004].

Horbowicz [2006], when examining dried and frozen foods obtained from shallots (*Allium cepa* var. *aggregatum*), showed that the content of quercetin decreased insignificantly with the passage of time. In the middle of the storage period (4 months), the author noted an increase in the content of quercetin in dried (by 4.8%) and frozen food (by

13.5%) compared to the measurement carried out 2 months earlier. In turn, after 8 months, there was a decrease by about 15% of quercetin in dried and 23% in frozen shallot. It has been shown [Horbowicz 2006] that quercetin in the shallot occurs mainly in the form of glucosides that are resistant to enzymatic degradation due to the limitation of the water content in the raw material or lowering the storage temperature.

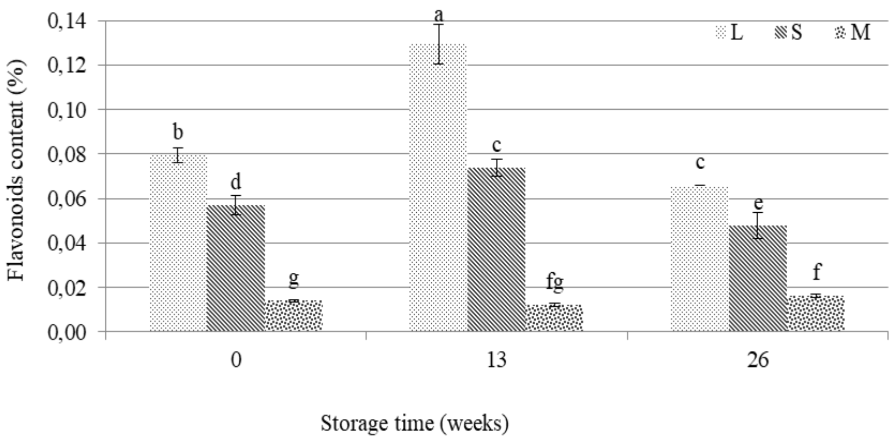

Fig. 2. Change in flavonoids content in blackthorn fruits depending on preservation method and storage time: L – freeze-drying, S – convection drying, M – freezing. Means followed by the same letter do not differ significantly at p = 0.05

The total polyphenol content determined on the basis of the Folin-Ciocalteu method was significantly differentiated both by the method of the raw material preservation and the storage time (Fig. 3). The analyzed parameter changed in a multi-directional manner with the time of storage of the raw material. The largest and, at the same time, systematic decrease in polyphenol content was recorded in lyophilized samples – by 38.32% in a 26-week period. However, in the case of the other two samples, an increase in the content of these compounds was observed between the first and the last measurement. Researchers dealing with the subject of polyphenols obtained equally diverse results. Misiak and Irzyniec [2009] showed that during the storage of lyophilized chokeberry fruits, the polyphenol content decreases. Piekut et al. [2016] described similar analogy in the case of frozen plant products. On the other hand, in convective dried material made from apples, that were stored at +25°C, it was shown that the polyphenol content is subject to complex changes. In the second week after preservation, it reaches the highest value, after which in the 12th week, there is a decrease in the amount of polyphenols, and then in the 20th week, their content increases to values close to the first measurements [Rząca and Witrowa-Rajchert, 2006].

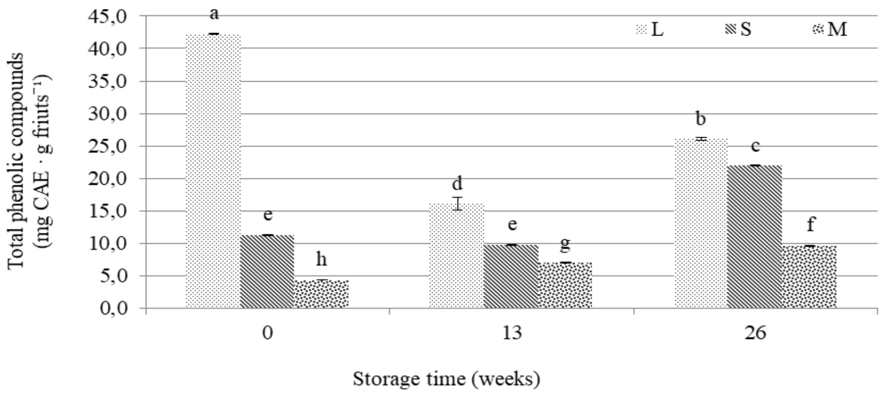

Fig. 3. Changes in the content of phenolic compounds in blackthorn fruits depending on preservation method and storage time: L – freeze-drying, S – convection drying, M – freezing. CAE – caffeic acid. Means followed by the same letter do not differ significantly at $p = 0.05$

The experimental factors used also significantly modified the ability to reduce iron ions (FRAP) (Fig. 4). The greatest reduction strength was recorded in lyophilized, non-stored samples. Despite the decrease in the reduction strength during storage, over time, freeze-dried products were characterized by the highest reduction activity compared to the other samples (S, M). Similarly to the total polyphenol content, the antioxidant activity of lyophilized and dried samples decreased in week 13. After subsequent 13 weeks of storage, there was a further decrease in the reduction strength of lyophilizates by nearly 55% (compared to the starting material), with a simultaneous increase in dried material (by 35%) and frozen foods (by 48%). Patthamakanokporn et al. [2008] reported that in the case of frozen guava fruits stored for 3 months, the analyzed FRAP parameter reached its highest value (73.1 μmol TE $\cdot$ g^{-1}) after one month of storage, then its decrease was recorded after 3 months of storage (57.5 μmol TE $\cdot$ g^{-1}).

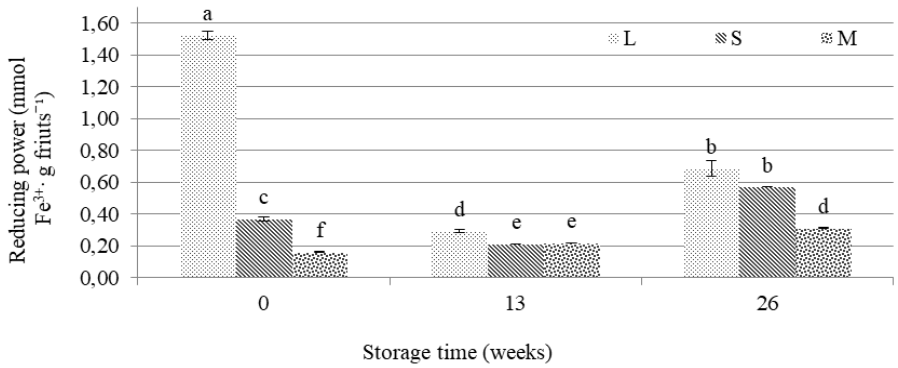

Fig. 4. Shaping the reducing power of blackthorn fruit in time depending on the method preservation and storage time: L – freeze-drying, S – convection drying, M – freezing. Means followed by the same letter do not differ significantly at $p = 0.05$

CONCLUSIONS

1. The analyzed methods of preservation and the storage time of the blackthorn fruit pulp modified the content of tested substances (anthocyanins, flavonoids, total polyphenols) and antioxidant activity.

2. In fruits subjected to the process of freeze-drying, with the passage of time, values of the analyzed parameters decreased, but nevertheless they remained at a higher level than in dried and frozen foods.

3. Among the preservation methods studied, lyophilization allowed to obtain a product with the most favorable storage parameters in terms of the content and activity of antioxidant compounds.

4. Comparing selected methods of preservation, it was found that with the passage of the storage time, the total polyphenol content and the ability to reduce iron ions increased in frozen samples.

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Streszczenie. Celem pracy było określenie wpływu wybranych metod utrwalania oraz czasu przechowywania na zawartość przeciwutleniaczy w owocach tarniny (*Prunus spinosa* L.). Utrwalone owoce przechowywano przez 13 i 26 tygodni bez dostępu światła w temperaturze pokojowej (próby liofilizowane i suszone konwekcyjnie) oraz w temperaturze –20°C (próby mrożone). Wykazano, że zarówno sposób utrwalania, jak i czas przechowywania istotnie wpływały na zawartość polifenoli (antocyjanów, flawonoidów, polifenoli ogółem) oraz właściwości przeciwutleniające (siła redukcji jonów Fe^{3+}) owoców tarniny. W miarę upływu czasu przechowywania notowano zmniejszenie zawartości antocyjanów we wszystkich wariantach utrwalania. W przypadku owoców mrożonych po 26 tygodniach przechowywania odnotowano wzrost zawartości flawonoidów (o 12,5%), polifenoli ogółem (o 48%) oraz zdolności do redukcji jonów żelaza (o 55%). W owocach suszonych po upływie tego okresu odnotowano zmniejszenie zawartości antocyjanów, wzrost zawartości polifenoli i siły redukcji. Największe wartości ocenianych parametrów po upływie założonego czasu przechowywania wykazano w liofilizatach.

Słowa kluczowe: *Prunus spinosa* L., antocyjany, flawonoidy, polifenole ogółem, aktywność przeciwutleniająca, utrwalanie, przechowywanie

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