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COMPOSITION AND ANTIOXIDANT PROPERTIES OF FRESH AND FROZEN STORED BLACKTHORN FRUITS (*PRUNUS SPINOSA* L.)

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ABSTRACT

Aim. Blackthorn (*Prunus spinosa* L.) is quite widespread bush occurring in Poland. Its fruits are easily available food products. The aim of this study was to investigate the basal chemical composition, especially antioxidant compounds in fresh and frozen stored blackthorn fruits.
Material and methods. Research material consisted of blackthorn fruits collected from the wild grown bushes, near Łącko. In the wash-out, dried and stone-loss fruits, the content of dry matter, protein, simple sugars, dietary fibre and ash was determined. The amount of antioxidant compounds was also tested: β-carotene, vitamin C, polyphenols, in this anthocyannins, as well as antioxidant activity expressed as an ability to quench free radical ABTS. Analyses were repeated in fruits stored in frozen state for three months.
Results. On the basis of the obtained results, it was observed that concerning basal chemical composition, the blackthorn fruits do not single out among other fruits from *Rosaceae* family. It was also reported that blackthorn fruits are rich in polyphenolic compounds, as well as in vitamin C, and those compounds, most probably contribute to their high antioxidant activity, being at the level of 43.6 μmol Trolox/g f.m. Storage in the freezing state reduced the contents of water, fat, dietary fiber and ash, but did not have significant impact on the other compounds.
Conclusions. Blackthorn fruits may constitute valuable source for preparations (tincture, wines, and teas), as well as an additive to other fruit processing, as a product of great pro-healthy properties. Freezing process and storage in that state did not significantly influence on nutritive and antioxidant compounds of blackthorn fruits. It may be therefore observed that several months of frozen storage is a good way to make them available for fruit processing and for the consumers, also the off-season.

Key words: blackthorn (*Prunus spinosa*), frozen storage, chemical composition, antioxidants, antioxidant activity

INTRODUCTION

At present, consumer awareness and interest in nutritional quality, especially in healthy food, is observed in many societies. They try to choose products with not only desired taste, but also those that may have a particularly positive impact on their health. These force the food manufacturers to develop new food products that will be attractive to the widest potential consumers and become competitive in the market. Nowadays, these actions can be divided into two streams: the first one is returning to natural, traditional products that are least processed, and the other one is a production of functional foods, often using unconventional materials or additives. Such materials can be wild grown fruits, which are often a good

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source of healthy compounds, as well as antioxidants. Among them, there is firstly presented a large group of polyphenolic compounds, but also vitamin C and E and β-carotene. Fruits of wild-grown bushes have been used for a long time in folk medicine, and processed by some housewives. Following current interest in unconventional food consumers, they are becoming increasingly important at the industrial scale.

One of rarely applied resources are blackthorn fruits (*Prunus spinosa* L.), which belong to the rose family (*Rosaceae*). Their common names include: blackthorn, grater, rasp or stickleback. They are closely related to the cultivated plums and cherries [Kucharska and Sokół-Łętowska 2008, Pelc et al. 2010]. Łuczaj and Szymański [2007] in Poland and Pardo de Santayana et al. [2007] in Spain mentioned the use of wild grown blackthorn fruits in their research reports. In addition, in many other countries, studies are carried out on different morphological parts of blackthorn, which are attributed to the medicinal properties. Due to the presence of flavonoids in blackthorn flowers, they have diuretic, laxative and may remove an excess of sodium ions from the body and harmful products of metabolism, reduce the permeability of blood vessels, as well as reduce an inflammation of the urinary tract. On the other hand, fruits may have slightly impressive, antibacterial and anti-inflammatory action [Pelc et al. 2010, Barros et al. 2010, Morales et al. 2013].

Despite all these positive properties, it is hard to find products of blackthorn fruits on the market or even with their addition. However, at the various types of portals dedicated to the natural medicine, a lot of interesting recipes associated with their use can be found. Among others, worth mentioning are jams made of them, juices and syrups recommended as an addition to tea, mousse from blackthorn and apple recommended for meat, wine and many more.

Seasonality of blackthorn fruits' ripening requires material processing after harvesting or storage under conditions that best preserve its good nutritional value. The best method for most plant sources is freezing and frozen storage.

The aim of this study was to determine the chemical composition of blackthorn fruits (*Prunus spinosa* L.) and investigation of the changes resulting from the storage of fruits for three months in a frozen state.

MATERIAL AND METHODS

Materials

The research material consisted of blackthorn fruits (*Prunus spinosa* L.) collected from wild grown bushes located in a mountain village, in the south of Poland, called Łącko. After collecting, the raw material was washed out and dried at room temperature, followed by removal of inedible parts – stones. The obtained laboratory probe was divided into two parts. One of them was frozen and frozen stored for three months at a temperature of –18°C, and the other one was dedicated to the current determinations.

Analytical methods

In the fresh material, the following analyses were determined: dry matter content by gravimetric method [AOAC 1995], sugars by the method of Luffa-Schoorl in the modification of Scalesa [Rutkowska (ed.) 1981] and vitamin C by xylene method, according to Tillmans in the modification of Pijanowski [Rutkowska (ed.) 1981].

The methanol extracts were prepared and then used to determine the content of total polyphenols, anthocyaninns [Benvenuti et al. 2004] and antioxidant activity [Re et al. 1999]. The remainder of the collected fruits underwent the process of freeze drying and the content of total protein was measured using Tecator Kjeltec 2200 (conversion factor N × 6.25), total ash [Rutkowska (ed.) 1981], fat amount using Tecator Soxtec 2050 Avanti, fiber content according to AOAC [1995] by using Tecator Fibertec system E and β-carotene [PN-90/A-75101/12].

The same analyses were repeated in the fruits after storage in the frozen state for a period of three months.

Statistical analysis

One-way analysis of variance was applied to estimate the changes in chemical composition and antioxidant activity in fresh and frozen stored blackthorn fruits. The significance of differences was estimated with Duncan's test at $p = 0.05$ (level of significance) with Statistica 7.1 software by StatSoft.

RESULTS AND DISCUSSION

The results of essential nutrients' contents in the edible part of fresh and frozen blackthorn fruits (*Prunus spinosa* L.) are shown in Table 1.

The dry matter content in fresh blackthorn fruits was at the level of 18.11%. This fruit, although well known in folk medicine, has not been a subject of many studies so far, hence in the literature there is little data with which one could compare the results. According to food commodity dictionary from 1957 [Ziemiański 1957], the dry matter content in blackthorn fruits was 30%, while in the flesh reached 17%. In the studies by Marakoğlu et al. [2005] the dry matter content reached 30.63% in fruits growing on the bushes in Turkey. Barros et al. [2010] showed 60.86% of moisture content in the whole fruits from the north-eastern area of Spain, while Ganhão et al. [2010] marked only 48.64% of moisture in the seeds-free fruits collected in Spain. These differences in dry matter content in blackthorn fruits may be related mainly to climatic conditions. Fruits growing in countries of warm and dry climate are characterized by lower water content. The obtained results are close to the dry weight, which were reported by Rejman [1987] and Hodun [2008] in Poland in various embodiments of prune. The frozen fruits had 16.09% of the dry weight, which is quite surprising.

Water is one of the first food components undergoing some changes during freezing. Its evaporation from the surface of the fruits reduces the mass of frozen fruits [Jarczyk and Berdowski 1999]. Ścibisz and Mitek [2007 b] concluded a significant increase in dry matter content after 4 months of frozen storage at –18°C of high bush variety of blueberries, but in the fruits stored at –35°C, the dry matter content changes were not statistically significant. Certainly, this process may depend on the type of packaging, in which the fruits were stored in a frozen state, as well as on the method of freeze-thaw. Leakage juice during defrosting can contribute to an increase in dry matter content.

The determined protein content in fresh fruits was 0.8 g per 100 g (4.42 g per 100 g d.m.). The value of 2.86 g/100 g d.m. was marked in Portuguese blackthorn fruits by Barros et al. [2010]. A similar result – 3.4 g per 100 g d.m. reported Marakoğlu et al. [2005] in the case of fruits harvested in southern Turkey. Almost twice higher protein content was designated by Ganhão et al. [2010] in the fruits of Spanish blackthorn. The obtained results are very similar to those presented by Hodun [2008] and concerning different varieties of plums, ranging from 0.6 to 0.8 g/100 g f.m. The protein content in the tested blackthorn fruits is also comparable to the content of this component in the other commonly consumed stone fruits such as: peaches – 1 g/100 g, cherries – 1 g per 100 g, apricots – 0.9 g/100 g, cherries 0.9 g per 100 g [Kunachowicz et al. 2005]. In the frozen stored fruits, the protein content was at the level of 0.34 g per 100 g (2.07 mg/100 g d.m.), while that difference was not statistically significant. Proteins are a food component, the contents of which are a changing little under the influence of frozen storage [Jarczyk and Berdowski 1999].

Table 1. Chemical composition of blackthorn fruits (*Prunus spinosa* L.)

Component	Fresh material		Frozen material	
	g/100 g f.m.	g/100 g d.m.	g/100 g f.m.	g/100 g d.m.
Dry matter	18.11 ±0.27^{a}	–	16.09 ±0.86^{b}	–
Protein	0.8 ±0.03^{a}	4.42 ±0.08^{a}	0.34 ±0.07^{a}	2.07 ±0.04^{a}
Fat	0.37 ±0.01^{a}	2.04 ±0.05^{a}	0.33 ±0.02^{b}	2.05 ±0.12^{a}
Sugars	5.52 ±0.23^{a}	30.48 ±1.27^{a}	5.0 ±0.1^{a}	31.07 ±0.62^{a}
Fiber	5.79 ±0.1^{a}	31.97 ±0.55^{a}	4.74 ±0.15^{b}	29.45 ±0.93^{a}
Ash	0.69 ±0.04^{a}	3.81 ±0.22^{a}	0.41 ±0.02^{b}	2.54 ±0.12^{b}

Mean with different letters in rows are statistically different at $p \leq 0.05$.

The fat content in the fresh fruits was 0.37 g per 100 g (2.04 g per 100 g d.m.), and in the frozen fruits was at the level of 0.33 g per 100 g (2.05 g per 100 g d.m.). Differences in the value calculated on dry matter were not statistically significant. The determined values are comparable with those published by Barros et al. [2010], Ganhão et al. [2010] and Morales et al. [2013]. Resulting values are within the range of 0.1 to 1 g per 100 g, which have been reported by Hodun [2008] and Kucharska and Sokół-Łętowska [2008] in the studies on different varieties of plums.

In the studied blackthorn fruits, the content of sugars was also measured. The quantity of them in fresh fruits was 5.52 g per 100 g, and in the frozen fruits reached the level of 5.0 g/100 g. They were respectively 30.48 and 31.07% of the dry weight of the fruit. In studies by Barros et al. [2010], the sugar content was 37.06% of the dry weight of blackthorn fruits, while the vast majority was glucose. Generally, the content of sugars in blackthorn fruits is small, also with regard to the fruits of other varieties of plums. Hodun [2008] and Rejman [1987] in their works marked an average sugar content in different varieties of plums in the range of 9 to 14% and from 15 to 17%, respectively. Precisely for this reason, they are fruits of strong acidic and tart taste. Their acidity was at 1.97% in the study by Marakoğlu et al. [2005], and 2.24% in the research by Ganhão et al. [2010]. This feature causes that blackthorn fruits do not belong to consumption fruits.

Barros et al. [2010] reported that total carbohydrates constitute 88.51% of the dry weight of blackthorn fruits. Except simple sugars, they comprise complex carbohydrates, including fiber. The tested material contained 5.79 g per 100 g (31.97 g/100 g d.m.) of fiber, and after frozen storage this content decreased significantly to 4.74 g per 100 g w.m. (29.45 g per 100 g d.m.). In the studies by Marakoğlu et al. [2005], blackthorn fruits contained fiber at the level of 4.6%.

Blackthorn fruits can be a good source of minerals. Their sum, determined as the residue after complete combustion of organic matter (ash) in the studied fruits was 0.69 g per 100 g (3.81 g per 100 g d.m.), and after frozen storage reached 0.41 g per 100 g (2.54 g per 100 g d.m.). These amounts are in the range of 0.3-0.8%, which is generally given for fruits [Kunachowicz et al. 2005], however the other authors marked higher ash contents in blackthorn fruits: Marakoğlu et al. [2005] – 2.72 g per 100 g, Ganhão et al. [2010] – 2.07%, and Barros et al. [2010] – 6.65 g/100 g d.m. Marakoğlu et al. [2005] investigated further that most abundant minerals presented in the blackthorn fruits are: K, Ca, P, Mg, Na, B and Al.

Table 2 presents the results of the analyses of antioxidant compounds contents and antioxidant activity in fresh and frozen blackthorn fruits.

Table 2. Content of antioxidants and antioxidant activity in blackthorn fruits (*Prunus spinosa* L.)

Component	Fresh material		Frozen material	
	mg/100 g f.m.	mg/100 g d.m.	mg/100 g f.m.	mg/100 g d.m.
Vitamin C	23.84 ±1.2^a	131.64 ±6.63^a	20.86 ±1.84^a	129.64 ±11.43^a
Polyphenols	599.2 ±15.6^a	3 308.67 ±86.14^a	539.5 ±17.7^b	3 353.01 ±11.0^a
Anthocyanins	71.75 ±3.6^a	396.19 ±19.88^a	66.78 ±0.92^a	415.04 ±5.72^a
	µg/100 g f.m.	µg/100 g d.m.	µg/100 g f.m.	µg/100 g d.m.
β-carotene	0.04 ±0.0^a	0.22 ±0.004^a	0.032 ±0.0^a	0.19 ±0.005^a
Antioxidant activity, µM Trolox/g f.m.	43.6 ±0.9^a	–	48.5 ±3.35^a	–

Mean with different letters in rows are statistically different at $p \leq 0.05$.

Vitamin C was determined at the level of 23.84 mg/100 g (131.64 mg/100 g d.m.) in the raw (fresh) material and 20.86 mg/100 g (129.64 mg/100 g d.m.) in the raw (frozen) material. The differences that occurred after period of frozen storage were not statistically significant, indicating well-selected temperature storage. The literature data confirms that the losses of this component in a storage temperature of –20°C can reach up to 10% [Jarczyk and Berdowski 1999].

The obtained values of vitamin C are in the range between 20 and 30 mg/100 g, which was provided by Kucharska and Sokół-Łętowska [2008]. Similar results at the level of 21.94 mg/100 g are presented by Jabłońska-Ryś et al. [2009]. Erturk et al. [2009] marked the content of vitamin C in juice of blackthorn fruits at the level of 3.8 mg/100 ml of juice. Smaller amounts of vitamin C (7.73 mg/100 g of fresh product) were reported by Morales et al. [2013] and Barros et al. [2010] – 15.69 mg/100 g d.m.

The tested fruits contained little amounts of β-carotene, at the level of 0.04 mg/100 g fresh product, what corresponded to a content of 0.22 mg/100 g d.m. Three times higher content of β-carotene – 0.78 mg/100 g d.m. was marked by Barros et al. [2010]. The fruit storage in the frozen state did not affect significantly the content of this component.

The total content of polyphenolic compounds, expressed as gallic acid (GAE) equaled 599.2 mg GAE/100 g of fresh fruits (3308.67 mg GAE/100 g d.m.) and 539.5 mg GAE/100 g of frozen fruits (3553.01 mg GAE/100 g d.m.). These values differed significantly among each other, while Ścibisz and Mitek [2007 a] showed no significant changes in total phenolics upon frozen storage of high bush variety of blueberries. A similar content – 623.48 mg/100 g was tagged in blackthorn fruits by Leja et al. [2007]. Slightly lower results were obtained by Erturk et al. [2009] – 407 mg GAE/100 g of f.m. and by Jabłońska-Ryś et al. [2009] – 402.67 mg GAE/100 g f.m. Similar amounts were presented by Stefãnut et al. [2011], but much lower values were obtained by Dragović-Uzelac et al. [2007]. The latter concerned blackthorn fruits harvested in October and November, while the contents of polyphenolic compounds in calculation on gallic acid were respectively: 54.656 mg/g and 85.875/100 g. Barros et al. [2010] marked in turn polyphenols in blackthorn fruits at the level of 83.4 mg GAE/g. These differences between the values given in the literature may arise not only from the diversity of biological material, but may also implicate from the determination methodology, particularly in relation to the extraction medium. Ganhão et al. [2010] marked the polyphenol content in aqueous, methanol and ethanol extracts of blackthorn fruits in the amount of 473, 326 and 134 mg GAE/100 g.

Ganhão et al. [2010] indicated that the content of polyphenolic compounds in blackthorn fruits consisted of procyanidins and catechins, hydroxycinnamic acid's derivatives and flavonols. According to Barros et al. [2010] flavonoids account for about one tenth of all polyphenolic compounds. All the authors pay attention to anthocyanins, which are the main pigments of blackthorn fruits. Their content in the presented studies was 71.75 mg/100 g (369.19 mg/100 g d.m.) of fresh fruits and 66.78 mg/100 g (415.04 mg/100 g d.m.) of frozen fruits. The observed difference was not statistically significant. Similarly Ścibisz and Mitek [2007b] did not show any significant changes in the content of anthocyanins as a result of frozen storage of high bush variety of blueberries. Very similar anthocyanins content – 66.9 mg/100 g – was marked in blackthorn fruits by Leja et al. [2007]. Slightly lower results were reported by Dragović-Uzelac et al. [2007] – in the fruits harvested in October, the anthocyanins content was 30.504 mg/100 g, and in November – 49.743 mg/100 g. A similar value of 41.3 mg/100 g f.m. was determined by Erturk et al. [2009]. According to Stefãnut et al. [2011] anthocyanins accounted for approximately one-tenth part of polyphenols in blackthorn fruits.

Antioxidant activity of blackthorn fruits, expressed as the ability to quench free radical ABTS, was 43.6 μmol Trolox/g f.m. in the case of fresh fruits. The value in frozen fruits was higher and equaled 48.5 μmol Trolox/g. The difference that occurred between fresh and frozen stored fruits was not statistically significant, but may indicate a greater availability of components as a result of cell's structure damage in the freezing process. In the study by Ścibisz and Mitek [2007 a], there was no significant effect of frozen storage of high bush variety of blueberries on their antioxidant activity.

Slightly lower values of total antioxidant activity of blackthorn fruits were received by Dragović-Uzelac

et al. [2007], amounting to 37.60 μmol Trolox/g in the case of fruit harvested in October and 38.56 μmol Trolox/g in fruits harvested in November, while Jabłońska-Ryś et al. [2009] reported antioxidant activity at the level of 5.33 μM TE/g f.m. Ganhão et al. [2010] determined antioxidant activity of blackthorn fruits in the aqueous, methanol and ethanol extracts, obtaining different values at a level of: 55.1, 35.9 and 7.1 μM TEAC/g f.m. Leja et al. [2007] examined the ability of blackthorn fruits to quench free radical $ABTS^{\cdot}$, giving a result in calculation to the percentage reduction RSA, amounting to 48.85%. Erturk et al. [2009] have set the total antioxidant activity of blackthorn fruits at the level of 78.99%.

CONCLUSIONS

Blackthorn fruits (*Prunus spinosa* L.), due to their organoleptic characteristics (bitter taste, high acidity) are not attractive food product. However, studies have confirmed that they are rich in components of antioxidant properties. Special attention is paid to their high vitamin C and polyphenols contents, including anthocyanins. Comparing identified and reported results on the content of total polyphenols in blackthorn fruits with the results published by Rupasinghe et al. [2006] and by Rop et al. [2009] concerning different cultivars or crosses of *Prunus species* in Europe, it can be concluded that polyphenol contents measured in our study are much higher, what also had an impact on higher antioxidant activity of these fruits. They are therefore a valuable source of large pro-healthy properties to receive infusions, wines, teas, or as an additive in the preparation of other fruits.

The process of freezing and frozen storage did not affect significantly the change in nutrients and antioxidants in blackthorn fruits. Noteworthy is the fact of the minimum, statistically insignificant reduction in antioxidant activity of fruits in the course of this process. It can therefore be considered that a few months frozen storage of blackthorn fruits is a good way to make them available for processing and for the consumers, also the off-season.

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SKŁAD I WŁAŚCIWOŚCI ANTYOKSYDACYJNE ŚWIEŻYCH I PRZECHOWYWANYCH ZAMRAŻALNICZO OWOCÓW ŚLIWY TARNINY (*PRUNUS SPINOSA* L.)

STRESZCZENIE

Cel. Tarnina (*Prunus spinosa* L.) jest krzewem dość rozpowszechnionym w naszym kraju, a jej owoce są produktem łatwo dostępnym. Celem podjętych badań było oznaczenie podstawowego składu chemicznego, ze szczególnym uwzględnieniem składników o właściwościach antyoksydacyjnych w świeżych owocach śliwy tarniny i przechowywanych przez kilka miesięcy w stanie zamrożonym.

Materiał i metodyka. Materiałem badawczym były owoce śliwy tarniny zebrane z dziko rosnących krzewów w okolicach Łącka. W umytych, osuszonych i wypestkowanych owocach oznaczono zawartość: suchej masy, białka, tłuszczu, cukrów prostych, błonnika i popiołu. Oznaczono także składniki o właściwościach antyoksydacyjnych: β-karoten, witaminę C, polifenole, w tym antocyjany, oraz całkowitą aktywność

antyoksydacyjną, wyrażoną jako zdolność wygaszania wolnego rodnika ABTS. Analizy powtórzono w owocach zamrożonych i przechowywanych w tym stanie przez trzy miesiące.

Wyniki. Na podstawie przeprowadzonych badań stwierdzono, iż w składzie podstawowym owoce tarniny nie wyróżniają się na tle innych owoców z rodziny różowatych. Wykazano również, że owoce śliwy tarniny są bogate w związki polifenolowe oraz witaminę C i najprawdopodobniej te składniki przyczyniają się do wyróżniającej je dużej aktywności antyoksydacyjnej określonej na poziomie 43,6 μmol Trolox/g ś.m. Przechowywanie zamrażalnicze istotnie wpłynęło na zmniejszenie zawartości wody, tłuszczu, błonnika i popiołu, natomiast nie miało istotnego wpływu na pozostałe składniki.

Wnioski. Owoce śliwy tarniny mogą być cennym surowcem na przetwory (nalewki, wina, herbatki) oraz dodatkiem do przetworów z innych owoców, o dużych właściwościach prozdrowotnych. Proces zamrażania oraz przechowywania w warunkach zamrażalniczych nie wpływa znacząco na zmianę zawartości składników odżywczych i antyoksydacyjnych w owocach tarniny. Można zatem uważać, że kilkumiesięczne przechowywanie owoców śliwy tarniny w warunkach zamrażalniczych jest dobrym sposobem na ich udostępnienie przetwórstwu owocowemu oraz konsumentom także poza sezonem.

Słowa kluczowe: śliwa tarnina (*Prunus spinosa*), przechowywanie zamrażalnicze, skład chemiczny, antyoksydanty, aktywność antyoksydacyjna

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