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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF BORAGE (*Borago officinalis* L.) SEEDS

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ABSTRACT

Recently the interest in borage seeds (mericarps) as an important source of γ-linolenic acid and a good natural antioxidant has increased, yet the knowledge about these properties of seeds developed by borage grown in Poland remains very scanty. Seeds collected from borage plants cultivated in the south-eastern region of Poland in the years 2017 and 2018 were characterized by following parameters: length – 5.0 mm, width – 2.7–2.8 mm, thickness 2.2 mm, weight of 1000 seeds – 17.2–19.4 g. The seeds contained 93.7% of dry matter, and in this 32.0% of lipids, 23.7% of protein and 1.1–1.8% of total sugars on average. Seventeen compounds were identified in the lipids with linoleic acid (35.1%), oleic + elaidic acids (20.8%), γ-linolenic acid (17.9%), palmitic acid (11.4%), stearic acid (5.3%), cis-11-eicosenoic acid (4.1%), and erucic acid (2.6%) as major ones. Other fatty acids constituted less than 2%. Saturated fatty acids constituted 17.5% of the total fatty acids. Flavonoids and phenolic acids accounted for 0.012% and 1.60–1.73% of seeds dry weight respectively. Antioxidant activity measured by FRAP assay and Foli Ciocalteau method accounted for 1225 μmol of ferrous equivalent Fe (II) and 29.7 mg of polyphenols per gram of seeds dry weight respectively. Using antiradical activity against DPPH radical following average parameters were obtained: T 50% – 49, DPPH rem – 73.7%, AE – 0.024 $dm^3 \cdot (\mu mol \cdot s)^{-1}$. 100 g of seeds d.w. contained 0.27–0.34 ml of essential oil. Content of four macroelements expressed as % of seeds d w. was following: P – 0.70, K – 0.48, Ca – 1.37 and Mg – 0.41.

Key words: fatty acids, protein, macroelements, polyphenols, DPPH, FRAP

INTRODUCTION

In Poland, borage is a rare annual plant occurring naturally as a self-seeding garden weed or cultivated for medicinal purposes [Pieszak et al. 2012]. It is seeded in April, flowers in Jun August and the most appropriate time for its seeds harvest is the first half of August [Suchorska and Osińska 1997c].

Borage flower is complete with one pistil of two-lobed superior ovary divided additionally with false septum into four parts [Polakowski 1994]. After fertilization, the ovary develops into an elongated schizocarp composed of 3 to 4 dark brown irregularly oblong and distinctly wrinkled mericarps [Szempliński 2017] which are usually called nutlets [Beaubaire and Simon 1987, Simpson 1993, De Haro et al. 2004] and sometimes also achenes [Suchorska and Osińska 1997]. In agricultural practice they are used as a sowing material and are commonly named seeds. Borage seeds ripe unsteadily and afterwards shatter easily, which makes them difficult to gather [Beaubaire and Simon 1987, Simpson 1993]. In the experiment carried out by Suchorska and Osińska [1997a], one- and two-year old dark brown, almost black seeds had an egg-oblong shape, the length of 4 to 7 mm, the width of 2–3 mm and thickness of 2–2.5 mm. In another experiment

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of these authors [Suchorska and Osińska 1997b], the weight of 1000 mature borage seeds varied from about 10 to 39 g depending on date of harvest and weather conditions. In the studies conducted by De Haro et al. [2002], 1000 seed weight of 206 cultivated and wild borage populations from different origins showed an overall range of variation from 9.3 to 26.3 g. In another study carried out by De Haro et al. [2004], 1000 seed weight of white flowered borage populations cultivated as vegetable in the North of Spain and of wild blue flowered roadside populations from the South of Spain amounted to 15.2 g and 14.1 g respectively and was lower than those of wild and cultivated north European populations (23.1 g). In Chile, weight of 1000 borage seeds measured by Berti et al. [2010] ranged from 12.3 g to 16.4 g depending on nitrogen rates. Characteristic part of borage seed is an elaiosome constituting about 6% of the whole seed weight [Del Río-Celestino et al. 2008].

Therapeutic properties of borage were already known in ancient times. Nowadays, borage is cultivated and collected from natural stands in several countries mainly as leafy vegetable and medicinal plant [Borowy et al. 2017]. Its importance as vegetable decreased after finding hepatotoxic alkaloid lycopsamine in the leaves [Larson et al. 1984]. On the other hand, significance of borage seeds as the richest plant source of γ-linoleic acid (GLA) increased [Beaubaire and Simon 1997], mainly owing to the fact that the borage seed oil does not contain alkaloids [Dodson. and Stermitz 1986]. GLA is a precursor of E_1 prostaglandin which is involved in regulating many metabolic functions [Fan and Chapkin 1998]. Numerous studies demonstrated that GLA had the capacity to relieve the signs and symptoms of several chronic inflammatory diseases, including rheumatoid arthritis and atopic dermatitis [Sergeant et al. 2016]. GLA can also be useful in gastrointestinal, respiratory and cardiovascular disorders [Gilani et al. 2007]. However, borage oil contains erucic acid as well, though usually in small quantities [De Haro et al. 2002] and this fatty acid is a risky food component [Knutsen et al. 2016]. Preliminary works aiming to breed borage types with high oil and GLA and low erucic acid content were undertaken by Galwey and Shirlin [1990] and De Haro et al. [2002].

Total fatty acids accounted for 26.4–31.9% of seed dry weight of mid-western American borage accession dependening on harvest time and GLA concentration in these fatty acids ranged from 18.1 to 20.0% [Beaubaire and Simon 1987]. Seeds of several English borage populations contained from 30.5 to 33.7% of oil and from 21.3 to 23.9% of GLA in the oil depending on flower colour and geographical origin [Galwey and Shirlin 1990]. In another experiment carried out in England, oil and GLA content ranged from 29.5 to 33.1% and from 18.5 to 21.8% respectively, depending on the crop stage and study year [Simpson 1993]. Seeds collected in northern Poland contained 34% of oil and in this 18% of GLA [Zadernowski et al. 1999]. In an experiment carried out in Chile by Berti et al. [1998], contents of oil in borage seeds and contents of GLA in the oil were dependent on maturity stage and varied from 31.5 to 33.1% and from 17.3 to 18.5% respectively. In another study conducted by Berti et al. [2002] in different locations in Southern Chile, content of oil in the seeds and GLA content in the oil varied from 31 to 32.4% and from 21.5 to 26.9%. Berti et al. [2010] stated no effect of N, P, K fertilization on seed oil content and fatty acid composition. According to these authors, composition of fatty acids depends mainly on air temperature during seed development and on seed maturity at harvest. In the studies conducted by De Haro et al. [2002] in Spain, oil content in borage seeds varied from 26.7 to 38.0% and GLA content in the oil varied from 8.7 to 28.6%. In another study conducted by De Haro et al. [2004], average oil content in the seeds of cultivated white flowered populations from northern Spain, wild blue flowered populations from southern Spain and wild and cultivated blue flowered populations from northern Europe were 34.5, 30,7 and 29.6%, and average GLA contents in the oil were 23.1, 18.9 and 20.4% respectively. Fully ripened seeds collected from the north-western region of Tunesia contained 79.2 mg of total fatty acids in 1 g of dry matter, while share of GLA in total fatty acids was 20.4%. Unsaturated fatty acids accounted for 84% of total fatty acids [Mhamdi et al. 2009]. GLA content in the oil obtained from borage seeds collected in Canada ranged from 23.2 to 30.0% depending on harvest date [El Hafid et al. 2002]. Remaining most important components of borage oil are linoleic (32.5–35.4%), oleic (17.7–24.2%), palmitic (10.2–10.7%) and stearic (4.2–5.6%) acids [Zadernowski et al. 1999, Mhamdi et al. 2009]. In the studies

conducted by Zadernowski et al. [1999] erucic acid accounted for 2.52% of total fatty acids while in the studies carried out by Galwey and Shirlin [1990] and De Haro et al. [2002, 2004] its mean contents ranged from 1.93% (white-flowered Spanish populations) to 2.80% (northern blue-flowered populations) and from 1.6% (white-flowered Spanish populations) to 3.7% (blue-flowered from northern Europe), respectively. Share of erucic acid in total fatty acids obtained by Berti et al. [2002] from seeds of Chilean borage population varied from 2.68% to 2.95% depending on seed maturity stage. Oil content and fatty acid composition in three seed fractions of different borage accessions studied by Del Río-Celestino et al. [2008] differed considerably. Endosperm + cotyledon dry matter contained 48.8% of oil on average, while seed coat and elaiosome contained only about 2.4% and 2.3% d.m. of oil respectively. GLA content in endosperm + cotyledon and in seed coat ranged from 13.7 to 14.8% d.m. and in elaiosome it was found at a very low level of 0.48% d.m. on average. Content of erucic acid in seed fractions studied ranged from 2.6 to 3.1% d.m. In the studies conducted by Galway and Shirlin [1990], GLA content was negatively correlated with oil content and positively correlated with erucic acid content. In the studies carried out by Mhamdi et al. [2009], saturated fatty acids of borage seed oil accounted for about 16% of total fatty acids.

Borage seeds also contain many volatile components, some of them with anti-microbial and therapeutic properties. Dry Tunesian borage seeds contained 0.1% of essential oil in which 16 compounds accounting for 88% of the whole oil and belonging to six chemical classes were identified by Mhamdi et al. [2009].

Apart from the above mentioned components, the seeds contain proteins, carbohydrates and phytates as major reserve compounds and in smaller quantities also other organic combinations. Moreover, seeds contain mineral elements and their content vary depending on plant species and their habitats [Duczmal and Tucholska 2000]. According to Budzyński and Zając [2010], 1 kg of seeds of four oleaginous plant species cultivated in Poland contain from 168.1 to 373.3 g of total protein, from 5.1 to 7.2 g of phosphorus, from 5.8 to 18.9 g of potassium and from 0.5 to 3.4 g of calcium. Content of total sugars in dehulled and defatted sunflower (*Helianthus annuus* L.) seeds determined by Balasaraswathi and Sadasivam [1997] was 7.08. Seeds of twenty soybean (*Glycine max* (L.) Merr.) genotypes evaluated by Hou et al. [2009] contained from 37.21 to 148.76 mg of total sugar in 1 g of seed dry matter. Up to the present, there have been no such data referring to borage. Berti et al. [2010] did not find significant effect of nitrogen fertilization on borage seed nitrogen and protein content although an upward trend was recorded as nitrogen rate increased.

Wettasinghe and Shahidi [1999, 2000] proved good antioxidant properties of borage seed meal and its extracts and attributed these effects to phenolic compounds, among which phenolic acids and flavonoids are of importance [Rice-Evans et al. 1997]. Wettasinghe et al. [2001] identified rosmarinic, sinapic, and syringic acids in the meal obtained from borage seeds of Canadian origin and total contribution of these acids in the meal was 0.617%. Zadernowski et al. [2002] identified 15 phenolic acids in the borage seeds collected in Poland and their total content amounted to 185.1 mg per kg of defatted seeds. Mhamdi et al. [2009] identified six phenolic acids in the fully ripened seeds of wild Tunesian borage populations and their total content amounted to 406.71 mg in 100 g of seed d.w. while content of total phenolic compounds amounted to 6.39 mg·1 g^{-1} d.w. In another study, Mhamdi et al. [2010] identified nine phenolic acids with rosmarinic, sinapic and syringic as the major ones and phenolic acid contents ranged from 2.45 to 10.98 mg·1 g^{-1} d.w depending on seed ripening stage. Antioxidant activity of borage seed extract did not depend on the high content of total phenolics but on the phenolic acid composition. Furthermore, total phenolic content observed at last stages of seed maturation coincided with the increase of temperature in the sampling region. Rice-Evans et al. [1997] revealed good antioxidant activity of flavonoids produced in different parts of plant but up to the present there has been no such information referring to borage seeds. Wettasinghe et al. [2001] stated absence of flavanoid compounds in borage seed meal and Gudej and Tomczyk [1996] stated occurrence of six flavonoid compounds in borage herb.

In recent years, interest in use of ground borage seeds or borage seed aqueous extract as natural antioxidants against lipid-protein oxidative deterioration in meat and meat products is evident [Sánchez-Escalante et al. 2003, Bellés et al. 2017, 2018]. Gómez-Estaca

et al. [2009] showed that incorporation of the extract obtained from ground borage seeds increased considerably antioxidant properties of some edible films.

The data presented above show that properties of borage seeds are dependent to some degree on their geographical origin whereas literature referring to seeds produced in natural conditions of Poland is scarce. Up to the present there has been no information about antioxidant potential of these seeds. The aim of this study was to determine chemical composition and antioxidant activity of borage seeds collected in south-eastern Poland. Additionally, basic morphological seed parameters were evaluated and measured.

MATERIALS AND METHODS

Physiologically mature borage seeds used in this study were collected by hand soon after dropping off on soil surface in the second half of July in the years 2017 and 2018. The seeds were produced by blue-flowering borage (*Borago officinalis* L.) plants cultivated in Felin Experimental Farm, University of Life Sciences in Lublin (215 m above sea level, 51°23'N latitude, 22°56'E longitude) In the first year, air temperatures were higher and sum of precipitation was lower than in the second year of study. Borage was grown in a warm place protected from wind, on podsolic soil developed from dusty medium loam containing 1.9% of organic matter and with pH (in H_2O) of 6.8. Seeds were seeded 1 cm deep in rows 80 cm apart at the rate of 6 kg·ha^{-1} on April 20, 2017 and April 24, 2018. Before seeding, following mineral fertilization was applied: 40 kg N (ammonium nitrate)·ha^{-1}, 30 kg P (triple superphosphate)·ha^{-1}, and 75 kg K (concentrated potassium salt)·ha^{-1}. Mature seeds were picked by hand five times in the period from July 11th to July 27th in 2017 and from July 16th to July 30th in 2018. After harvest, the seeds were kept in a ventilated room of 25°C temperature for two months and then they were packed in plastic bags and kept in the temperature of 5°C. Seed colour and shape, structure of seed coat, length, width and thickness of seeds, as well as weight of 1000 seeds were determined in both years in March. In the same month content of phosphorus (spectrophotometric method), potassium (flame photometry), calcium (flame photometry) and magnesium (atomic absorption spectrometry) in the seeds was determined at the Regional Chemical Agricultural Station in Lublin. In April, seeds dry weight, content of protein, total sugars, flavonoids, as well as content of lipids and their composition were determined in the Central Research Laboratory of the University of Life Sciences in Lublin. Over the same period of time content of essential oil and content of phenolic acids in the seeds were determined in the Laboratory of Vegetables and Herb Raw Material Quality and seeds antioxidant activity was determined in the Laboratory of Herb Raw Material Quality Evaluation.

Seeds dry weight was determined using oven method (drying at 105°C to constant weight) [PN-A--79011-3:1998]. Content of proteins was determined by the Kjeldahl method and content of total sugars – by the Luff Schoorl method [Charłampowicz 1966]. Content of oil was determined by the Soxhlet method (hot extraction with hexane) [PN-EN ISO 734:2016-03]. Fatty acid composition was established with a gas chromatograph after initial saponification of lipids and esterification of acids according to AOAC 969.33 and 963.22 norms [2000]. Detailed procedure was previously described by Borowy et al. [2017]. Content of essential oil was determined using direct steam distillation [Polish Pharmacopoeia V, 2002], content of phenolic acids (caffeic acid equivalent) – using spectrometric Arnova method [Polish Pharmacopoeia 1999], and content of flavonoids (quercetin equivalent) – using spectrophotometric method [Polish Pharmacopoeia VI 2002].

The antioxidant potential of borage seeds was determined using the FRAP assay (measurement of iron ion reduction ability) [Benzie and Strain 1966], using Folin Ciocalteu method (measurement of polyphenolics content) [Singelton and Rossi 1965, Prior 2005] and the free radical method with DPPH reagent [Brand-Williams et al. 1995]. The seeds were ground in electric WZ-1 seed grinder for 5 min. The ground meal was mixed with water at 90°C and then left for 30 min. before analysis began. Detailed procedure was described by Borowy et al. [2017]. For the first method the results were calculated in FRAP units: µmol of ferrous equivalent Fe (II) per gram of seeds dry weight. Standard curve was made using aqueous solution of $FeSO_4 \cdot 7\ H_2O$). For the second method the results were calculated in mg of polyphenols per gram of seeds dry weight and then recalculated onto caffeic acid. In the

Fig. 1. Mericarps of borage (phot. M. Niedziółka)

third method, the kinetics of DPPH radical reduction was determined and following parameters were measured: content of remaining unreduced DPPH radical (DPPH rem) expressed as % of total DPPH content, time (.) necessary for 50% diminution of initial radical concentration and antiradical efficiency (AE) expressed in $dm^3 \cdot (\mu mol \cdot s)^{-1}$.

Seed parameters were measured in four replications and chemical analyses were carried out three times. Results referring to seed parameters, to main seed components as well as to antioxidant seed potential were analyzed statistically by analysis of variance (ANOVA) involving a model appropriate for bifactorial experiments and orthogonal data, while the differences between means were estimated by Tukey's test at the P = 0.05 level of significance.

RESULTS

Borage plants grew up to 80–90 cm high. First flowers appeared on June 10th in 2017 and on June 16th in 2018. The flower bloomed one day, than the corolla dropped off and a flower succeeding in the inflorescence opened its corolla within the next one – two days. This way flowering continued till end of July in 2017 and till beginning of August in 2018. Flowers were visited by a great number by pollinating insects. First ripe seeds dropped off on July 5–9th. Seeds in all stages of development were found on the plant simultaneously and ripe seeds abscised easily, which made them difficult to collect. The dropped seeds, lying on soil surface, rich in lipids, protein, and to a much lesser degree, in sugars (Tab. 3) were readily eaten by animal pests. In the middle of June, shoots of several borage plants were invaded by thistle aphid (*Brachycaudus cardui* L.) and caterpillars of painted lady (*Vanessa cardui* L.).

In both study years, collected seeds were black with slight brownish tint. The seeds were oblique, inversely egg-shaped, narrowed on top and sharp ended. Seed coat was hard with numerous distinct ribs arranged along mericarp. On abdominal site, middle rib was distinctly bigger and ended with sharp top of the mericarp. In lower part of some mericarps, several ribs were arranged diagonally. Moreover, there were tiny papillae on lower parts of some mericarps. Place of mericarp attachment to floral disc was surrounded with elliptic transversely wrinkled fold ("collar"), about 0.5 mm high formed from pericarp inside of which there was light–brown elaiosome (Fig. 1), up to 2 mm long. It is worth pointing out that directly after seed dropping the elaiosomes were brightly white. Seeds from the year 2017 were 4.0–6.0 mm long, 2.0–3.0 mm wide and 1.8–2.5 mm thick and for the year 2018 those parameters made 4.0–6.0 mm, 2.0–3.5 mm and 1.9–2.5 mm respectively. Mean 1000 seeds weight ranged from 17.1 g in 2017 to 19.4 g in 2018. All parameters of the seeds harvested in 2018 were bigger, however the only significant differences were those in seed weight and seed width (Tab. 1).

In the borage seeds dry weight, phosphorus accounted for 0.70–0.78%, potassium accounted for 0.68–1.00%, calcium accounted for 0.86–1.17% and magnesium accounted for 0.3%–0.41%. Total content of these mineral elements ranged from 2.60 to 3.36% of seeds d. w. Contents of potassium and calcium were significantly dependent on the year of study (Tab. 2).

Depending on the year, borage seeds contained from 93.6 to 93.8% of dry matter in which lipids and protein were the main components accounting for 33.94–34.29% and 22.73–24.63%, respectively. Share of total sugars was much lower and ranged from 1.08 to 1.84% with the differences being significant. During steam distillation, a yellowish essential oil was obtained and its share in seeds dry weight (0.27–0.34%) differed significantly in dependence on the year (Tab. 3). Content of phenolic acids made 1.50% in the first year and 1.62% in the second year with the differ-

Table 1. Length, width, thickness and 1000 seeds weight of borage seeds in the years 2017 and 2018

Feature	2017		2018		$LSD_{0.05}$
	min.–max.	mean ±SD	min.–max.	mean ±SD	
Length (mm)	4.50–6.10	4.96 ±0.65	4.60–6.20	5.10 ±0.69	n.s.
Width (mm)	2.50–3.60	2.70 ±0.48	2.50–3.90	2.80 ±0.62	0.011
Thickness (mm)	2.06–2.68	2.23 ±0.29	2.09–3.10	2.24 ±0.41	n.s.
1000 seeds weight (g)	17.11–17.25	17.18 ±0.06	19.32–19.49	19.43 ±0.08	0.91

Table 2. Content of four macroelements in borage seeds in the years 2017 and 2018 (% d.w.)

Year	P	K	Ca	Mg
2017	0.70	0.68	1.17	0.41
2018	0.78	1.00	0.86	0.36
$LSD_{0.05}$	n.s.	0.116	0.253	n.s.

Table 3. Proximate composition of borage seeds in the years 2017 and 2018

Component	2017		2018		$LSD_{0.05}$
	min.–max.	mean ±SD	min.–max.	mean ±SD	
Dry matter (% f.w.)	93.3–94.3	93.8 ±0.6	93.2–94.0	93.6 ±0.5	n.s.
Lipids/oil (% d.w.)	27.3–36.9	34.29 ±5.0	27.1–36.4	33.94 ±4.9	n.s.
Protein (% d.w.)	19.9–26.1	24.63 ±3.4	18.2–22.9	22.73 ±2.7	n.s.
Total sugars (% d.w.)	1.59–1.90	1.84 ±0.16	0.91–1.11	1.08 ±0.10	0.010
Essential oil (ml·100 g^{-1} d.w.)	0.31–0.38	0.34 ±0.04	0.25–0.30	0.27 ±0.03	0.041

Table 4. Content of flavonoids and phenolic acids in borage seeds in the years 2017 and 2018

Component	2017		2018		$LSD_{0.05}$
	min.–max.	mean ±SD	min.–max.	mean ±SD	
Flavonoids (% d.w., quercetin equivalent)	0.010–0.014	0.012 ±0.002	0.010–0.014	0.012 ±0.001	n.s.
Phenolic acids (% d.w., caffeic acid equivalent)	1.53–1.69	1.62 ±0.04	1.49–1.51	1.50 ±0.01	0.098

ences being signifcant. Content of flavonoids was the same in both years of study accounting for 0.012% of seed dry weight only (Tab. 4). Seeds harvested in 2017 were smaller but they contained significantly more total sugars, essential oil and phenolic acids than those collected in 2018. Content of other components did not depend on harvest year (Tabs. 3 and 4).

Seventeen fatty acids were determined in borage seed oil and their content was dependent to a small degree on the year of study (Tab. 5). Main components of the oil were following fatty acids: linoleic (accounting for 35.06% of oil on average), γ-linolenic (17.93%), palmitic (11.39%), stearic (5.31%), gondoic (4.07%), erucic (2.59%) and nervonic (1.59%). Contents of oleic and elaidic acids were determined together and made 20.79% of oil on average. Share of each from eight remaining fatty acids was less than 0.4%. Saturated fatty acids constituted 17.5% of the total fatty acids. Omega 3 (α-linolenic), omega 6 (linoleic + γ-linolenic + eicosadienoic) and omega 9 (oleic + gondoic + erucic + nervonic) fatty acids accounted for 0.14%, 53.14% and 29.03% of the total fatty acids on average, respectively. Content of fatty acids calculated as percent of seeds fresh weight was more than three times lower retaining similar dependences between individual components (Tab. 5).

Table 5. Fatty acid composition of borage seed lipids in the years 2017 and 2018

Fatty acids	% of the total fatty acids in the oil			g·100 g^{-1} seeds f.w.		
	2017	2028	mean	2017	2018	mean
Tetradecanoic (myristic)	0.07 ±0.01	0.07 ±0.01	0.07 ±0.01	0.020	0.022	0.021
Hexadecanoic (palmitic)	11.48 ±2.54	11.29 ±2.50	11.39 ±2.52	3.690	3.587	3.639
cis-9-Hexadecenoic (palmitoleic)	0.17 ±0.01	0.15 ±0.01	0.16 ±0.01	0.050	0.048	0.049
Heptadecanoic (margaric)	0.06 ±0.01	0.07 ±0.01	0.065 ±0.01	0.020	0.021	0.021
Octadecanoic (stearic)	5.25 ±1.02	5.36 ±1.04	5.305 ±1.03	1.691	1.703	1.697
cis-9-Octadecenoic + trans-9-octadecenoic (oleic + elaidic)	20.89 ±1.97	20.69 ±1.95	20.79 ±1.96	6.720	6.572	6.646
cis-9,12-Octadecadienoic (linoleic)	35.16 ±10.68	34.96 ±10.61	35.06 ±10.65	11.311	11.105	11.208
cis-6,9,12-Octadecatrienoic (γ-linolenic)	17.60 ±0.26	18.26 ±0.27	17.93 ±0.27	5.661	5.801	5.731
cis-9,12,15-Octadecatrienoic (α-linolenic)	0.14 ±0.06	0.14 ±0.05	0.14 ±0.06	0.051	0.043	0.047
Eicosenoic (arachidic)	0.36 ±0.11	0.35 ±0.11	0.355 ±0.11	0.121	0.111	0.116
cis-11-Eicosanoic (gondoic)	4.11 ±1.39	4.03 ±1.37	4.07 ±0.38	1.320	1.280	1.300
cis-11,14-Eicosadienoic	0.15 ±0.01	0.14 ±0.01	0.145 ±0.01	0.050	0.044	0.047
Docosanoic (behenic)	0.22 ±0.03	0.22 ±0.03	0.22 ±0.03	0.070	0.070	0.070
cis-13-Docosenoic (erucic)	2.63 ±0.52	2.54 ±0.50	2.585 ±0.51	0.851	0.805	0.828
Tetracosanoic (lignoceric)	0.10 ±0.01	0.11 ±0.02	0.105 ±0.02	0.031	0.033	0.032
cis-15-Tetracosenoic (nervonic)	1.60 ±0.07	1.57 ±0.06	1.585 ±0.07	0.511	0.497	0.504
Saturated fatty acids	17.54	17.46	17.50	5.641	5.547	5.594
Monounsaturated fatty acids	29.39	28.97	29.18	9.450	9.202	9.326
Polyunsaturated fatty acids	53.05	53.49	53.27	17.060	16.994	17.027
Omega 3 (α-linolenic)	0.14	0.14	0.14	0.051	0.043	0.047
Omega 6 (linoleic + γ-linolenic + eicosadienoic)	52.91	53.36	53.14	17.011	16.951	16.981
Omega 9 (oleic + gondoic + erucic + nervonic)	29.23	28.82	29.03	9.401	9.155	9.27

Table 6. Antioxidant activity of borage seeds in the years 2017 and 2018

Method and unit of measurement	2017		2018		$LSD_{0.05}$
	min.–max.	mean ±SD	min.–max.	mean ±SD	
FRAP (μmol Fe II·g^{-1} seeds d.w.)	1227–1249	1241 ±16	1151–1240	1191 ±48	49.9
Folin-Ciocalteu (mg plyphenols·g^{-1} seeds d.w.)	30.3–32.2	31.4 ±1.0	27.6–28.2	27.9 ±0.4	3.26
T(s)50% (%)	38.0–38.0	38.0 ±0.0	54.0–68.0	60.0 ±7.3	5.46
DPPH (DPPH rem, %)	69.1–75.9	73.4 ±3.8	70.4–79.9	74.0 ±4.9	n.s.
AE (dm^3·[μmol s^{-1}])	0.033–0.034	0.033 ±0.001	0.014–0.016	0.015 ±0.001	0.0127

The measurements of iron reduction ability, polyphenols content and DPPH radical reduction showed good antioxidant potential of borage seeds in both study years with the indication of higher potential in 2017 (tab. 6). An average ferric reducing ability, polyphenols content and antiradical efficiency of seeds harvested in 2017 were significantly higher with the time necessary for 50% reduction of initial DPPH radical concentration being significantly shorter in comparison to the seeds from the year 2018. Content of remaining unreduced DPPH radical was similar in both years.

DISCUSSION

Description of borage seed morphology available in Polish literature is simplified [Suchorska and Osińska 1997a, Szempliński 2017]. In the present work, the seed morphology was described according to Kulpa [1988], who studied seeds of thirteen Boraginaceae species occurring in Poland yet putting borage seeds aside. Taking into consideration the size as well as the development of the "collar" surrounding the elaiosome, appearance of the borage seed was most similar to the seeds of common comphrey (*Symphytum officinale* L.), small bugloss (*Anchusa arvensis* (L.) M. Bieb.), common bugloss (*Anchusa officinalis* L.) and monkswort (*Nonnea pulla* (L.) DC.) [Kulpa 1988], some of them considered by De Haro et al. [2004] as a possible source of GLA.

Literature data show that origin of borage seeds has some influence on their properties. Weight of 1000 seeds collected in the present experiment (Tab. 1) was situated in the ranges determined by Suchorska and Osińska [1997b] and De Haro et al. [2002] and was higher than that of Spanish [De Haro et al. 2004] and Chilean [Berti et al. 2010] borage populations. Larger seeds make harvest and oil extraction easier [Galwey and Shirlin 1990]. Data illustrating the length, width and thickness of borage seeds are in line with those presented by Suchorska and Osińska [1997c]. Significant differences in seed weight and width between study years can be explained by differentiated weather conditions [Duczmal and Tucholska 2000].

Comparing the contents of studied macroelements in borage seeds (Tab. 2) with those in seeds of twelve cultivated plant species characterized by Duczmal et Tucholska [2000], it can be stated that borage seeds are rich in mineral constituents. Content of phosphorus in borage seeds was lower than in the seeds of sunflower and rye (*Secale cereale* L.), similar to that in the seeds of flax (*Linum usitatissimum* L.) and wheat (*Triticum aestivum* L.) and higher than in the seeds of eight other species. It was similar to the content of phosphorus in the seeds of four oleaginous plants presented by Budzyński and Zając [2010]. In the case of potassium, seeds studied contained this macroelement in a lower quantity than the seeds of horse chestnut (*Aesculus hippocastanum* L.), sunflower, broad bean

(*Vicia faba* L. var. *major* Harz.), narrow-leaved vetch (*Vicia sativa* ssp. *angustifolia* (L.) Gaudich.) and pea (*Pisum sativum* L.), in a similar quantity as the seeds of yellow lupine (*Lupinus luteus* L.) and in a higher quantity than the seeds of six other species. Content of potassium in borage seeds was similar to that in the seeds of linum, sunflower and rapeseed (*Brassica napus* L. ssp. *oleifera* Metzg.) presented by Budzyński and Zając [2010] and much lower than in soybean seeds. Content of calcium was considerably higher than in the seeds of all other species discussed by Duczmal and Tucholska [2000] and by Budzyński and Zając [2010]. For example it was about 3.6 times higher than in flax seeds, 6.8 times higher than in sunflower seeds and more than 100 times higher than in barley (*Hordeum vulgare* L.) seeds. Content of magnesium in borage seeds was similar to that in the seeds of flax and sunflower and considerably higher than in the seeds of ten other species mentioned by Duczmal and Tucholska [2000].

Content of dry matter in borage seeds stated in this experiment (tab. 3) was on a very similar level to that in the seeds of four other oleaginous plants cultivated in Poland [Budzyński and Zając 2010]. Content of seed lipids and share of GLA in the lipids were very close to those determined by Zadernowski et al. [1999] in the seeds of northern Polish borage population. Comparing those data with the results obtained in other countries it can be stated that the lipids content was on a high level, while GLA content was on a low level [Beaubaire and Simon 1987, Galwey and Shirlin 1990, Simpson 1993, Berti et al. 1998, 2002, 2010, De Haro et al. 2002, 2004, El Hafid et al. 2002, Mhamdi et al. 2009]. This is consistent with the results obtained by Galwey and Shirlin [1990] showing negative correlation between these two components. Differentiated content of lipids and GLA can be explained by differences in borage genotype [Galwey and Shirlin 1990, De Haro et al. 2002, 2004], seeding and harvest time [Beaubaire and Simon 1987, Simpson 1993, Berti et al. 2002, El Hafid et al. 2002], seed maturity stage [Berti et al. 2002], and growing conditions, among others by differences in latitude [Berti et al. 2002] as well as in rainfall and air temperatures [El Hafid et al. 2002]. Seeds studied in the present experiment were at physiological maturity and according to Berti et al. [2010] at this stage content of oil in borage seeds increases.

Content of erucic acid was very approximate to that stated by Zadernowski et al. [1999]. It was also similar to erucic acid content in the seeds of northern English blue flowered borage populations [Galwey and Shirlin 1990] and slightly lower than in the Chilean borage seeds [Berti et al. 2002] but higher than in the seeds of majority of Spanish blue and white flowered populations [De Haro et al. 2002, 2004]. Content of saturated fatty acids was in line with that stated by Mhamdi et al. [2009]. Composition of borage seed oil determined in the present experiment was very similar to that stated by De Haro et al. [2002] and Del Río-Celestino et al. [2008] in Spain. It differed considerably from the composition of oil obtained from borage flowers and leaves by Borowy et al. [2017] in the same natural conditions.

Content of protein (Tab. 3) was comparable with that in the seeds of sunflower, flax and rapeseed and much lower than in soybean seeds [Budzyński and Zając 2010]. Borage seeds studied in this experiment contained considerably less total sugars than the soybean and sunflower seeds analysed by Balasaraswathi and Sadasivam [1997] and Hou et al. [2009]. Content of phenolic acids determined in the present study by means of spectrophotometer with Arnova reagent (Tab. 4) was much higher than that stated by Wettasinghe et al. [2001], Zadernowski et al. [2002] and Mhamdi et al. [2009, 2010], who used more precise methods. It was also much higher than the phenolic acids content in borage flowers and leaves determined by Borowy et al. [2017]. However, it was in line with high total polyphenols content determined in this experiment by Folin Ciocalteu method (Tab. 6). Content of flavonoids in borage seeds studied was much lower than that determined in oil obtained from borage flowers and leaves by Borowy et al. [2017]. No other data referring to flavonoids content in the seeds or in borage plant were found in the literature. Content of essential oil in seeds dry weight determined in the present experiment was considerably higher than that stated by Mhamdi et al. [2009] in the seeds of wild Tunesian borage population. Seeds studied were produced by plants grown in good agrotechnical conditions, which favoured production of bioactive compounds [Kołodziej 2018].

Data presented in the literature obtained by different analytical procedures and showing high an-

tiradical potential of borage seeds [Wettasighe and Shahidi 1999, 2000, Mhamdi et al. 2010] are in principle consistent with the results of the present experiment. According to the classification proposed by Sánchez-Moreno et al. [1998], antiradical efficiency of studied borage seeds can be defined as very high. It was much higher than that of borage flowers and leaves developed by plants cultivated in the same natural conditions and determined using the same analytical methods by Borowy et al. [2017]. Ferric reducing capacity of water infusion obtained from borage seeds in the present experiment was much higher than that of the sole and catfish gelatin films incorporated with borage seed extract by Gómez-Estaca et al. [2009].

CONCLUSIONS

During two years' study, physiologically mature borage seeds (mericarps) produced by plants cultivated in good agrotechnical conditions in south-eastern Poland were oblique inversely egg-shaped and characterized by hard seed coat of black colour with slight brownish tint, longitudinal ribbing, and up to 2 mm long elaiosome surrounded with elliptic "collar". The seeds were rather big and rich in macroelents, especially in calcium. They contained a lot of proteins and little sugars. Content of lipids in the seeds was high while content of GLA in the seed oil was low whereas content of erucic acid was on a medium level. Content of total phenolics and phenolic acids in seed dry matter was high while content of flavonoids was very low. In both study years, antioxidant activity of seed meal water infusion was high. Seeds collected in a warmer and dryer year were smaller but contained more sugars, total phenolics, phenolic acids and essential oil as well as their antioxidant activity was higher. Recapitulating concusion is the statement that properties of studied borage seeds did not differ considerably from those stated in other countries.

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