Evaluation of polar polyphenols with antioxidant activities in *Papaver somniferum* L.

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Summary

The presence of total polyphenols (free and bound) of polar fractions and their antioxidant activities was evaluated in twelve commercial poppy cultivars with different colours of seeds (blue, grey, white and ochre). Seeds of blue poppy cultivars with the lowest oil content (432 $g \cdot kg^{-1}$) had the highest content of total polar polyphenols (~289.9 $mg \cdot kg^{-1}$ plant material) and highest antioxidant activities determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and the ferric reducing antioxidant power (FRAP) methods (337.5 $mg \cdot kg^{-1}$ and 488.5 $mg \cdot kg^{-1}$ plant material, respectively). White poppy seeds had the lowest polyphenol content (~194.8 $mg \cdot kg^{-1}$ plant material) and lowest antioxidant activities determined by DPPH and FRAP methods (196.7 $mg \cdot kg^{-1}$ and 401.0 $mg \cdot kg^{-1}$ plant material, respectively). Ochre and grey poppy cultivars had comparable contents of above mentioned compounds. Polar and non-polar compounds of homogenized poppy seeds increased oxidative stability of sunflower oil, ranging from 4.7 h to 9.2 h. Our results suggest that oil stabilization is mediated by substances other than non-polar compounds. Thus, the presence of polyphenols with antioxidant activity in poppy residues after oil extraction should be considered for the applications as possible additives for oil stabilization.

Keywords

Papaver somniferum; seed extracts; antioxidant activity; polar fractions; oil stability

The poppy (*Papaver somniferum* L.) is an annual herb, member of the family Papaveraceae. In addition to its use as a foodstuff in manufacturing of bakery products and edible oil, poppy is interesting thanks to its contents of several medically important secondary metabolites, members of the large and diverse group of benzylisoquinoline alkaloids. The main alkaloid of poppy is morphine, which occurs in the latex of aerial organs [1], especially in poppy capsule and straw. Poppy seeds are included in the diet in several countries, in particular in the Central and Eastern Europe. Poppy has been improved in long-term breeding programs and many cultivars with different colour of seeds such as white, silver-grey, grey-blue, dark-blue, brown, ochre and black were registered in several countries. Blue poppy seeds are the most marketable from the perspective of consumers and food manufacturers. Poppy seeds contain high amounts of nutrients, including proteins (18–20 %), saccharides (16–24 %), fibre (5–8 %), minerals (6 %), particularly calcium [2], vitamins (particularly vitamin E) [3], as well as oils (42–57 %) [4]. Cold-pressed poppy oil contains a high amount of polyunsaturated fatty acids (PUFAs) [5]. Poppy seed oil is used also as a stabilizer to prevent deterioration of foods by oxidation due to the antioxidant potential of several substances found in oil [3]. PUFAs are considerably unstable against oxidation caused by oxygen free radicals [6], while autooxidation is responsible for the formation of toxic compounds [7]. However, antioxidants present in the seeds play a protective role in oxidative processes by free radical-scaveng-

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ing properties [8]. Excellent antioxidant capacity of hydrophilic polar components from different plants was previously reported [9–11]. Also, the antioxidant capacity of olive oil after 12 months of storage was linked to the content of minor polar phenolic components [12]. After oil extraction, more polar antioxidants remain in the press cake [13] and are not significantly destructed during the oilseed processing procedure. Press cakes collected after cold-pressing of oilseeds are a good source of proteins (forming 21–28 %, w/w) and are usually used for animal feeding [14]. However, they may also be a potential source of biologically active substances beneficial to human health. Content of antioxidant compounds in the form of simple phenols or flavonoids in press cakes depends on the type of seeds, location of cultivation, climate, harvest time, as well as solvents and conditions used in an extraction procedure [15]. Antioxidant compounds are also present in defatted oilseeds in free and/or bound forms [16]. Presence of bound polyphenols was observed in oilseeds of flax [17] and cotton [18]. A minor part of the press cake contains lignans and their degradation products [19].

The objective of the present study was to evaluate the oil content in poppy cultivars from Slovakia, presence of free and bound polyphenols in them, and potential antioxidant activities of the extracts of poppy seeds after oil extraction. The presence of free and bound polyphenols from selected defatted poppy seeds with different colours was compared using high-performance liquid chromatographic (HPLC) fingerprints. The ability of poppy seeds, after oil extraction, to stabilize model oil and to prevent oil oxidation was also tested. To the best of our knowledge, this is the first study on phenolic substances and antioxidant activities of poppy residues after the oil-extraction process, and on their potential ability to stabilize model oils.

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), gallic acid, formic acid, methanol for HPLC and ferric chloride were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Methanol, *n*-hexane, sodium carbonate, ethyl acetate, acetic acid, sodium acetate, hydrochloric acid (analytical grade) and Folin-Ciocalteu reagent were purchased from Mikrochem (Pezinok, Slovakia).

Plant material

Twelve poppy (*Papaver somniferum* L.) cultivars originating from four countries (Slovakia, Czech Republic, Hungary, and Austria) were cultivated in the year 2015 in field trials in Vígľaš-Pstruša (Slovakia). Samples included poppy cultivars with different colours of the seeds, namely, Albin (originating in Slovakia) and Racek (originating in Czech Republic) with white seeds, Redy (originating in Czech Republic) with ochre seeds, Malsar (originating in Slovakia) with grey seeds, Aristo (originating in Austria), Bergam (originating in Slovakia), Buddha (originating in Hungary), Gerlach (originating in Slovakia), Major (originating in Slovakia), Maraton (originating in Slovakia), Opal (originating in Slovakia) and Orfeus (originating in Czech Republic) with blue seeds.

Seed homogenization and oil extraction

Mature seeds of poppy cultivars were homogenized by Ultra-Turrax Tube Drive control (IKA, Staufen im Breisgau, Germany) and then centrifuged at 5000 ×*g* during 10 min (Himac CT6E, Hitachi Koki, Tokyo, Japan). Homogenized samples (1 g each) were defatted by extraction with 100 ml of *n*-hexane during 24 h at laboratory temperature. After the extraction, *n*-hexane fraction was decanted, solvent was evaporated and weight of the residual oil was determined. Defatted poppy seeds were dried and used for the extraction of free and bound polyphenols.

Extraction of polyphenols

Extraction of free polyphenols

Defatted and dried samples of poppy seeds were extracted by methanol (1 g of defatted seed samples in 100 ml of methanol) during 24 h at laboratory temperature. The mixture was then centrifuged at 5000 ×*g* using the Mini Spin Microcentrifuge (Eppendorf, Hamburg, Germany) during 10 min, methanol was evaporated and the residue solubilized in 0.5 ml of methanol. These free phenolic fractions were used for analysis of free polyphenol content (*PC*) and determination of antioxidant activities by photometric and HPLC analysis.

Extraction of bound polyphenols

Solid defatted residues of poppy seeds after the extraction of free polyphenols (samples of 1 g each) were used for bound polyphenol extraction with 100 ml of 10 $g \cdot l^{-1}$ Na_2CO_3 during 24 h at laboratory temperature. Then, the mixture was centrifuged at 5000 ×*g* using Eppendorf Mini Spin Microcentrifuge during 10 min and pH of the supernatant was adjusted to 2.0 with 1 $mol \cdot l^{-1}$

HCl. Subsequently, 10 ml of ethyl acetate was added, the mixture was shaken and centrifuged at 5 000 ×*g* during 10 min. Ethyl acetate fraction was evaporated and the residues were solubilized in methanol (0.5 ml). These bound phenolic fractions were used for analysis of bound *PC* and determination of antioxidant activities by photometric and HPLC analysis.

Photometric analysis

Polyphenol content (*PC*) in poppy seed extracts (free or bound phenolic fractions) was measured by the method of Singleton and Rossi [20] using the Folin-Ciocalteu reagent. The sample (40 μl) was mixed with 40 μl of the Folin-Ciocalteu reagent and with 400 μl of sodium carbonate (200 g·l^{-1}), and then centrifuged at 10 000 ×*g* using Eppendorf Mini Spin Microcentrifuge for 10 min. The supernatant (240 μl) was used for determination of *PC* at 690 nm using ELx800 Absorbance Reader (BioTek, Winooski, Vermont, USA). Gallic acid (25–100 mg·l^{-1}) was used as a standard. *PC* was expressed as milligrams of gallic acid equivalent (GAE) per kilogram of plant material.

Antioxidant activities of samples containing free or bound phenolic fractions were measured by the DPPH and the ferric reducing antioxidant power (FRAP) methods. The radical-scavenging activity by DPPH method [21] was determined in the reaction containing extract (25 μl) and 100 μl of DPPH solution (0.012 g DPPH in 100 ml methanol). Absorbance was determined at 540 nm using ELx800 Absorbance Reader. Trolox (10–100 mg·l^{-1}) was used as a standard and results were expressed as milligrams of Trolox equivalents (TE) per kilogram of plant material.

FRAP method [22] was performed using the reaction mixture containing 35 μl extract, 265 μl mixture of 0.3 mol·l^{-1} acetate buffer (pH 3.6), 10 mmol·l^{-1} TPTZ and 20 mmol·l^{-1} $FeCl_3$ mixed in a ratio 10 : 1 : 1 (v/v/v). The mixture was incubated during 5 min at 37 °C and absorbance was determined at 600 nm using ELx800 Absorbance Reader. Trolox was used as a standard and results were expressed as milligrams of TE per kilogram of plant material.

HPLC analysis

HPLC analysis of free or bound phenolic fractions was carried out for selected poppy cultivars with different colours (Major, Malsar, Racek and Redy). The Agilent 1200 Series HPLC System (Agilent Technologies, Santa Clara, California, USA) consisting from a binary pump, diode-array detector SL detector, degasser and the column temperature controller was used for analysis. The system was controlled and data processed using Agilent ChemStation software Rev. B.04.03 (Agilent). The chromatographic separation was performed in the Eclipse XD8-C18 column (3.0 mm × 100 mm, particle size 3.5 μm; Agilent) using water solution of formic acid (A; 99 : 1, v/v) and methanol solution of formic acid (B; 99 : 1, v/v) as the mobile phase at a flow rate of 0.7 ml·min^{-1}. The gradient program was set as follows: 0–5 min, 0–20 % B; 5–45 min, 20–95 % B; 45–60 min, 95 % B. Detection was carried out at a wavelength of 256 nm. The column temperature was maintained at 30 °C and the injection volume of each sample and of the standard solution was 10 μl. The HPLC mobile phase was prepared daily, filtered through a membrane filter (pore size 0.45 μm) and degassed before use.

Rancimat method

The influence of non-polar and/or polar compounds of the poppy seeds on stabilization of model oil (sunflower oil) was evaluated using 743 Rancimat for oils and fats (Metrohm, Herisau, Switzerland). Homogenized seeds containing non-polar and polar compounds, and homogenized defatted poppy seeds containing polar compounds were used and the oxidation process was carried out in a reaction vessel at 100 °C under air flow of 10 l·h^{-1}. The reaction vessel contained either 0.5 g of homogenized seeds or 0.5 g of defatted homogenized seeds, both with 2.5 g of sunflower oil. Volatile oxidation products formed during oxidation were collected in a vessel containing 60 ml of deionized water (conductivity ≤ 0.1 μS·cm^{-1}) and water conductivity was monitored continuously. A sudden change in electrical conductivity of water was noticed in the induction period point, indicating the launch of the oxidation process.

Statistical analysis

Experimental data were evaluated by Statgraphic Centurion XV (Statpoint Technologies, Warrenton, Virginia, USA). Pearson's correlation was used for analysis of correlation between values, and Fisher's least significant difference (LSD) test was used for the comparison of significant differences. All determinations were carried out in triplicates.

RESULTS AND DISCUSSION

Characterization of poppy cultivars

The content of oil in poppy seeds, the polyphenol content and the antioxidant activities of free and bound phenolic fractions from defatted seeds

of 12 evaluated poppy cultivars are presented in Tab. 1. The content of oil ranged within cultivars from 406 g·kg^{-1} (Malsar) to 502 g·kg^{-1} (Redy). Since all cultivars had been grown under the same conditions (location, climate, year) and harvested at the same time, the differences in the oil content were taken as affected by the poppy cultivar. LANČARIČOVÁ et al. [23] analysed the oil content in the same 12 poppy cultivars grown in the same location (Vigľaš-Pstruša, Slovakia) four years before (in 2011). The oil content determined in our samples (grown in 2015) was approximately 2.4 times lower than that of LANČARIČOVÁ et al. [23]. The differences could have been caused by weather conditions in the years 2011 and 2015. Nevertheless, the highest oil content was in the ochre and the white seeds in both studies. The cultivars Redy (ochre) and Racek (white) had the highest content of oil while the cultivars Major and Buddha (both blue) had the lowest content of oil. The seeds with high oil content (about 500 g·kg^{-1}) are more interesting for oil production than sunflower seeds with 275 g·kg^{-1} or flax seeds with 336 g·kg^{-1} of oil content [3]. According to Federal Institute for Risk Assessment [24], white poppy seeds contain more oil than blue or grey ones. Our results showed that the highest oil content was in ochre seeds followed by white, grey and blue seeds (average values 502 g·kg^{-1}; 442 g·kg^{-1}; 436 g·kg^{-1} and 432 g·kg^{-1}, respectively).

PC in free phenolic fractions obtained after oil extraction from poppy seeds, as well as their antioxidant activities metasured by the DPPH and FRAP methods, were determined. Methanol extraction of defatted seeds yielded highest amounts of free polyphenols with blue cultivars, namely, Major, Maraton and Orfeus. The values of this parameter significantly varied in the range from 157.2 mg·kg^{-1} to 260.9 mg·kg^{-1} of plant material (Tab. 1). Data on *PC* in poppy seeds were previously reported [25, 26]. However, those results cannot be directly compared to ours because, in those studies, the *PC* was measured in whole oilseeds. Oilseeds contain mainly lipophilic antioxidants in oils, but also polar antioxidants in other seed compartments [9]. Therefore, methanol extraction without elimination of lipophilic compounds allows a higher recovery of phenolic compounds (442.2 mg·kg^{-1} of plant material [25]) than that with elimination of lipophilic compounds (Tab. 1).

The antioxidant activities of free phenolic fractions of defatted poppy seeds were investigated by the DPPH and FRAP methods. The blue seeds of the cultivar Major had significantly higher both *DPPH* and *FRAP* antioxidant activi-

Tab. 1. Oil content in poppy seeds, free and bound polyphenol contents, and antioxidant activities of methanol extracts from defatted poppy seeds.

Cultivar	Oil content [g·kg^{-1}]	Free compounds			Bound compounds		
		PC [mg·kg^{-1}]	*DPPH* [mg·kg^{-1}]	*FRAP* [mg·kg^{-1}]	*PC* [mg·kg^{-1}]	*DPPH* [mg·kg^{-1}]	*FRAP* [mg·kg^{-1}]
Bergam	433 ± 5^{bc}	225.8 ± 4.3^{fg}	298.3 ± 0.4^{e}	386.2 ± 15.8^{abc}	50.6 ± 3.5^{cd}	44.3 ± 1.0^{i}	50.1 ± 5.2^{c}
Gerlach	429 ± 9^{bc}	236.5 ± 12.6^{gh}	323.4 ± 1.7^{f}	355.5 ± 16.6^{a}	57.5 ± 2.1^{de}	37.8 ± 0.3^{fg}	59.5 ± 3.9^{de}
Major	406 ± 5^{a}	260.9 ± 8.7^{i}	365.8 ± 7.3^{g}	501.4 ± 7.8^{f}	55.2 ± 2.6^{de}	38.0 ± 1.8^{fg}	58.1 ± 4.1^{cde}
Malsar	436 ± 8^{c}	209.8 ± 3.5^{de}	271.8 ± 8.1^{d}	379.4 ± 18.4^{ab}	46.5 ± 5.3^{bc}	27.1 ± 1.7^{d}	33.4 ± 1.8^{b}
Maraton	437 ± 2^{c}	246.0 ± 3.5^{h}	332.5 ± 6.9^{f}	427.6 ± 0.2^{cd}	59.5 ± 4.0^{e}	34.5 ± 0.6^{e}	68.5 ± 2.1^{fg}
Opal	450 ± 5^{d}	201.9 ± 2.2^{cd}	227.9 ± 6.9^{b}	410.6 ± 6.9^{bcd}	56.1 ± 1.7^{de}	37.7 ± 0.9^{fg}	59.7 ± 3.7^{def}
Orfeus	449 ± 1^{d}	258.4 ± 0.9^{i}	302.4 ± 7.5^{e}	424.6 ± 16.7^{cd}	59.3 ± 0.6^{e}	41.1 ± 1.2^{h}	72.2 ± 6.6^{g}
Aristo	427 ± 4^{bc}	210.8 ± 6.8^{de}	278.6 ± 3.6^{d}	438.5 ± 15.0^{de}	59.6 ± 0.4^{e}	37.2 ± 1.3^{f}	70.2 ± 3.5^{g}
Buddha	423 ± 2^{b}	223.7 ± 2.7^{f}	257.9 ± 0.7^{c}	470.3 ± 4.8^{ef}	57.7 ± 1.2^{de}	42.2 ± 0.2^{h}	55.5 ± 7.5^{cd}
Albin	424 ± 5^{b}	157.2 ± 2.2^{a}	150.0 ± 8.7^{a}	378.2 ± 10.8^{ab}	29.5 ± 1.2^{a}	15.3 ± 0.2^{b}	16.4 ± 2.0^{a}
Racek	460 ± 14^{d}	175.8 ± 14.6^{b}	227.6 ± 2.3^{b}	391.3 ± 10.0^{abc}	27.1 ± 1.3^{a}	0.4 ± 0.0^{a}	16.1 ± 2.5^{a}
Redy	502 ± 12^{e}	219.7 ± 8.8^{ef}	280.1 ± 7.1^{d}	442.4 ± 1.3^{de}	39.9 ± 2.3^{b}	17.3 ± 1.0^{c}	17.8 ± 0.7^{a}

Mean values followed by the same small letter in superscript are not statistically different ($p < 0.05$) as measured by the Fisher's LSD test.

The oil content is expressed as gram of oil per kilogram of poppy seeds. *PC* – polyphenol contents, expressed as milligrams of gallic acid equivalents per kilogram of plant material; Antioxidant activities are expressed as milligrams of Trolox equivalents per kilogram of plant material. *DPPH* – antioxidant activity measured by 2,2-diphenyl-1-picrylhydrazyl radical-scavenging method, *FRAP* – antioxidant activity measured by ferric reducing antioxidant power method.

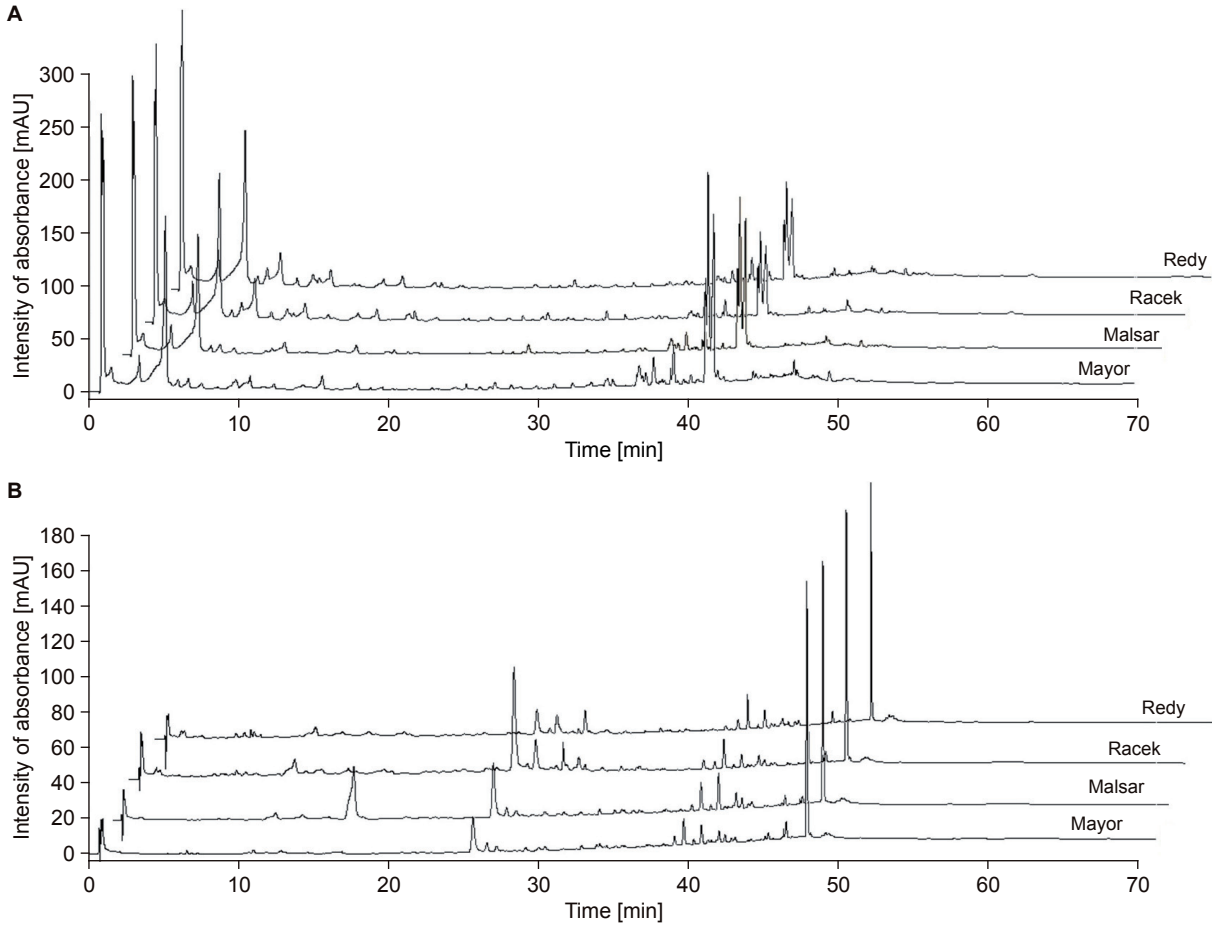

Fig. 1. HPLC profiles of polyphenol-containing extracts from seeds of selected cultivars of various colours.
A – free polyphenol compounds, B – bound polyphenol compounds.

ties than the seeds of other cultivars (260.9 mg·kg^{-1} and 365.8 mg·kg^{-1} of plant material, respectively). Generally, antioxidant activities of free phenolic fractions determined by the FRAP method were higher than those determined by the DPPH method (Tab. 1). The DPPH method is sensitive to steric hindrance and different antioxidants can react at different rates [27]. Our results suggested that the seeds of poppy cultivars with higher *PC* exhibited higher antioxidant activities in free phenolic fractions than those with lower *PC*. Some authors studied antioxidant activities in poppy seed extracts [25, 26, 28], but it is difficult to directly compare the results obtained in different investigations. Half maximal effective concentration (EC_{50}) values of the poppy extracts measured by the DPPH method varied in the range of 0.05–224.6 mg.ml^{-1} of the poppy extract [25, 26, 28]. Chan et al. [26] found that the extract from *P. somniferum* L. had the lowest *PC*, but the highest radical-scavenging activities among different tested spices. Rasheed et al. [28] similarly found that poppy extracts had the highest antioxidant activities among the other tested plant extracts. In the present study (Tab. 1), the total content of free polyphenols and antioxidant activities of defatted poppy cultivars increased in the order: white < grey < ochre < blue seeds.

PC in bound phenolic fractions obtained after the extraction of free polyphenols from defatted poppy seeds, as well as their antioxidant activities measured by the DPPH and FRAP methods, were determined. Similar to free *PC*, the blue seeds (cultivars Aristo, Maraton and Orfeus) had the highest content of bound polyphenols (59.3–59.6 mg·kg^{-1} of plant material). White (cultivars Racek and Albin) and ochre seeds (cultivar Redy) had lower *PC*, ranging from 27.1 mg·kg^{-1} to 39.9 mg·kg^{-1}. Bound *PC* in defatted poppy seeds was lower in comparison with free polyphenols and accounted for 15–28 % of their amounts (Tab. 1). Several authors reported data on bound *PC* in dif-

ferent oilseeds [17, 18, 29]. Similar to poppy seeds, defatted seeds of camelina and sophia had lower content of bound polyphenols than that of free polyphenols [29]. To the best our knowledge, the presence and quantity of bound polyphenols in poppy seeds has not been published.

The antioxidant activities of bound phenolic fractions of defatted poppy seeds were investigated by the DPPH and FRAP methods. The highest *DPPH* antioxidant activity linked to bound compounds was observed in blue seed cultivars Bergam, Major, Gerlach and Opal. Other blue cultivars Orfeus, Aristo and Maraton expressed the highest *FRAP* antioxidant activities of bound compounds. On the contrary, the white and ochre seed cultivars (Albin, Racek and Redy) had the lowest antioxidant activities (determined by DPPH and FRAP methods) of bound phenolic fractions, although seeds of cultivars Redy and Racek had the highest oil content. The antioxidant activities of bound phenolic fractions were lower than those of free phenolic fractions (Tab. 1). A similar trend was observed in a previous study where antioxidant activities of free phenolic fractions of defatted camelina and sophia seeds were lower than those of bound phenolic fractions [29]. The total content of bound polyphenols as well as *DPPH* and *FRAP* antioxidant activities increased in the order white < ochre < grey < blue seeds.

Agro-industrial plant by-products were widely investigated for their potential repeated use [9]. Poppy (*P. somniferum* L.) seeds were suggested as a potential source of usable antioxidants due to the high content of total polyphenols [26]. In the present study, we showed that poppy seeds after oil extraction contained significant amounts of biologically active substances and, therefore, they can be considered as functional additives for foods or beverages.

Characterization of poppy seeds by HPLC

Liquid chromatography is able to provide comprehensive and quantitative chemical characterization of plant extracts [30, 31]. HPLC analysis of defatted seed extracts with the highest free and bound PC from selected poppies with different colour of seeds was carried out for cultivars Major (blue seeds), Malsar (grey seeds), Racek (white seeds) and Redy (ochre seeds) (Fig. 1). Gradient elution was applied. Only the peaks with a relatively high intensity were selected to the characteristic profiles. The number of detected peaks varied from 89 to 108 for the free compounds in various cultivars. Only 39–78 peaks were of bound polyphenols. Significant variation in free and bound compounds among the cultivars was observed (Fig. 1). The similarity of HPLC profiles of free and bound compounds was evaluated by the Pearson's correlation of areas of peaks with high intensity (more as 1 % of the total area of detected compounds) (Tab. 2). HPLC profiles of free phenolic fractions exhibited high similarity for all tested cultivars (Fig. 1A). This finding suggested the presence of similar compounds in all poppy extracts at different contents. Low Pearson's correlations were found for bound phenolic fractions of the poppy cultivars (Tab. 2). The bound phenolic fractions from defatted poppy seeds were prepared by reactive extraction. Therefore, the bound compounds were present in a modified form and their contents were less comparable (Fig. 1B) as those of free compounds.

Influence of poppy seeds on oil stability

The oilseeds after oil extraction can be potentially incorporated as functional ingredients in food, beverages or feed due to the presence of biologically active substances. Free and bound compounds with antioxidant activity present in plants are able to prevent oxidation of oil during maturation and storage [32, 33]. These properties possess non-polar as well as polar compounds with antioxidant capacities. Therefore, we tested the potential of defatted homogenized poppy seeds containing polar compounds to prevent the oxidation of model oil by the Rancimat method. Homogenized poppy seeds containing non-polar and polar compounds were used as a control (Fig. 2). Generally, homogenized poppy seeds used as control increased oxidative stability of sunflower oil in the

Tab. 2. Pearson's correlation of HPLC profiles of free and bound phenolic fractions from seeds of selected cultivars of various colours.

	F1	F2	F3	F4
F1	–			
F2	0.8986*	–		
F3	0.8897*	0.7996*	–	
F4	0.7821*	0.7087*	0.7365*	–

	B1	B2	B3	B4
B1	–			
B2	0.6519*	–		
B3	0.5408*	0.5807*	–	
B4	0.4774*	0.2088	0.0440	–

F1–F4 – free phenolic fraction, B1–B4 – bound phenolic fraction. 1 – cultivar Major (blue seeds), 2 – cultivar Malsar (grey seeds), 3 – cultivar Racek (white seeds), 4 – cultivar Redy (ochre seeds). * – correlation coefficient with $p < 0.05$.

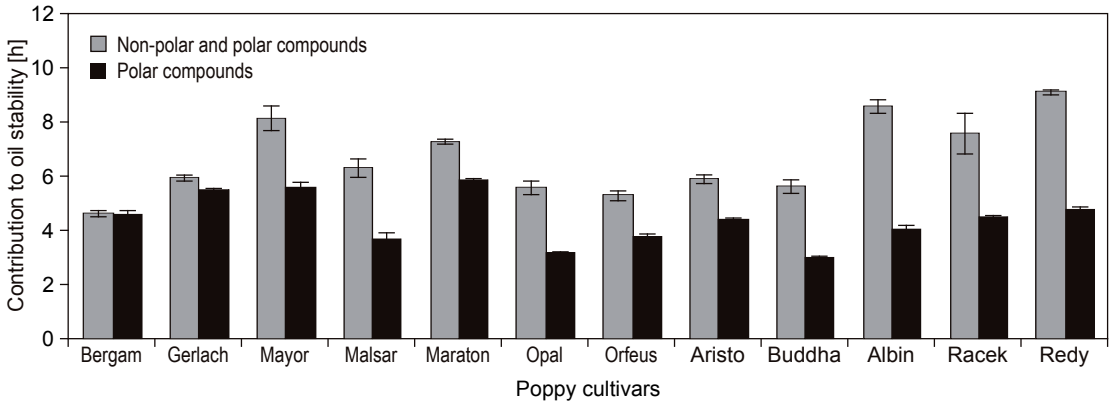

Fig. 2. Contribution of non-polar and polar compounds contained in homogenized poppy seeds, and polar compounds in defatted homogenized poppy seeds, on oxidative stability of model oil.

range from 4.7 h (Bergam) to 9.2 h (Redy). The oil stability was slightly decreased after oil extraction from homogenized poppy seeds, ranging from 3.0 h (Opal) to 5.9 h (Maraton). Non-polar compounds present in homogenized poppy seeds contributed to oil stability in the range of 0.9–31.5 %, while polar compounds present in defatted poppy seeds were able to prevent the oxidation of sunflower oil and to increase its oxidation stability in the range of 68.5–99.1 % for blue seed cultivars Bergam, Gerlach, Mayor, Maraton, Orfeus and Aristo. These cultivars showed the highest *PC* and antioxidant activities measured by the DPPH and FRAP methods in the free and bound phenolic fractions (Tab. 1). The results presented in Fig. 2 suggest that the polar compounds are mainly responsible for the oil stability in the case of certain cultivars with blue seeds. The presence of polar antioxidants in oilseeds is extremely important because they combat lipid oxidation in its initial stages. They are fundamental for the protection of lipid oxidation at low contents [34]. TERPINC et al. [35] reported that the methanolic extracts from defatted oilseeds were able to prevent the oil oxidation. Defatted white mustards with the highest *PC* and antioxidant activities measured by the DPPH method were able to stabilize model oil effectively for 14 days. The highest differences in the contri-

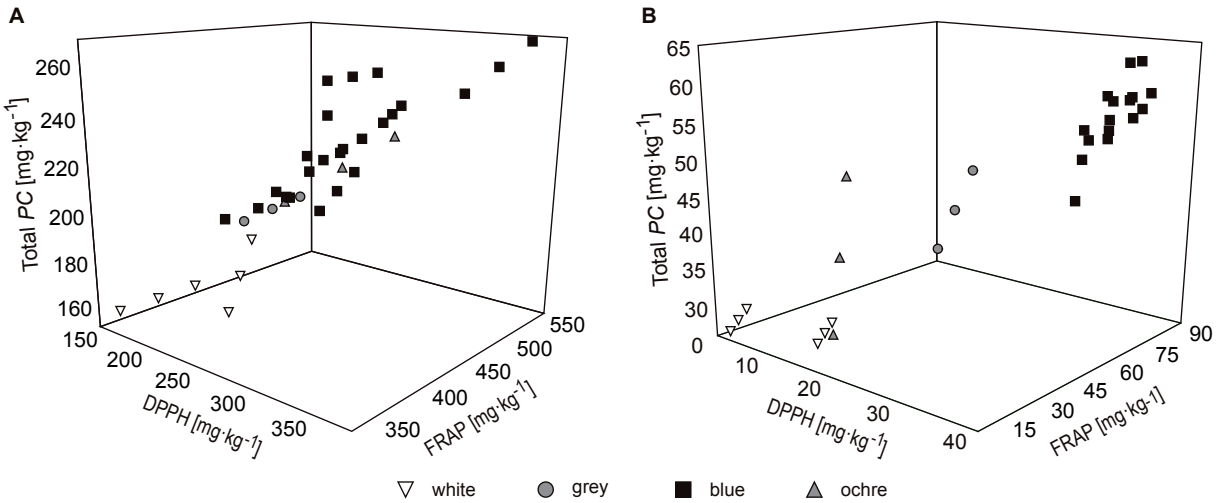

Fig. 3. Comparison of total polyphenols content and antioxidant activities of phenolic fractions of defatted poppy seeds of different colours.

A – free polyphenol compounds, B – bound polyphenol compounds.
PC – polyphenol contents, expressed as milligrams of gallic acid equivalents per kilogram of plant material. Antioxidant activities are expressed as milligrams of Trolox equivalents per kilogram of plant material.
DPPH – antioxidant activity measured by 2,2-diphenyl-1-picrylhydrazyl radical-scavenging method, *FRAP* – antioxidant activity measured by ferric reducing antioxidant power method.

bution of non-polar and polar compounds to the model oil stability were observed in white, ochre and grey seed cultivars (Fig. 2). The contribution to stability of non-polar (41.9–53.2 %) and the polar compounds (46.8–59.3 %) present in white (Albin, Racek), ochre (Redy), grey (Malsar) and selected blue poppy seeds (Opal, Buddha) was comparable (Fig. 2). These cultivars exhibited the lowest *PC* and antioxidant activities in the free and bound phenolic fractions (Tab. 1), except of the cultivar Buddha. Polyphenols and their content can vary depending on genotype (cultivar) and climatic conditions during growth of poppy plants [36]. These results suggest that the cakes of selected blue seed cultivars, after oil recovery, can be used as oil stabilizers. The cakes of oilseeds, after oil recovery, are currently not efficiently utilized, being rather used as animal feed, together with cakes obtained from other seeds [37].

Correlation analysis

Correlation analysis was performed by comparing of free or bound PC and antioxidant activities of free or bound compounds determined by the DPPH and FRAP methods (Fig. 3). The best correlations were observed between free polyphenols and antioxidant activities of free compounds measured by the DPPH method ($R^2 = 0.92$; $p < 0.05$), and for bound polyphenols and antioxidant activities of bound compounds determined by the DPPH method ($R^2 = 0.88$, $p < 0.05$) and by the FRAP method ($R^2 = 0.91$, $p < 0.05$). No significant correlation was observed between free polyphenols and antioxidant activities determined by the FRAP method. The reason can be that the DPPH and FRAP methods are based on different reaction mechanisms. The antioxidant activities of the free compounds measured by the DPPH and FRAP methods did not correlate with each other, as can be partially observed in Tab. 1. This suggests than some free compounds are not able to scavenge DPPH radicals and to reduce ferric ions at the same time. Similarly, TERPINC et al. [35] did not confirm the relationship between total *PC* and antioxidant activities determined by the DPPH method in oil cake extracts (camellia, linseed and rapeseed). On the other hand, GANGOPADHYAY et al. [38] found that the antioxidant activity determined by the DPPH and FRAP methods correlated with total polyphenols extracted from barley (0.98 and 0.96, respectively). It is not always possible to conclude that the polyphenols are responsible for antioxidant activities, but our findings (Fig. 1B) suggest that the antioxidant activities were directly linked to the *PC* (free or bound) in poppy seed extracts. Moreover, the correlation analysis between free or bound polyphenols vs the ability of non-polar or polar compounds to increase the model oil stability determined by the Rancimat method was carried out. There was no significant correlation between antioxidant activities determined by in vitro methods and thermal stability of the oil (data not shown).

CONCLUSIONS

The present study demonstrated that seeds of poppy cultivars contain biologically active substances in free and bound phenolic fractions. The highest contents of free and bound polyphenols, as well as the highest antioxidant activities, were determined for extracts from poppy cultivars with blue seeds. White poppy seeds (Albin, Racek) had the lowest content of polyphenols and the lowest antioxidant activities. HPLC profiles showed variation in free and bound compounds from poppy seeds with different colours. Free phenolic fractions of selected cultivars with different colours evaluated by HPLC showed high similarity to each other. Defatted poppy seeds were able to stabilize a model oil and prevent its oxidation. Defatted poppy seeds with a higher content of polyphenols (free and bound) and with higher antioxidant activities were able to stabilize the model oil more effectively than those with lower contents of polyphenols and with lower antioxidant activities although, by correlation analysis, no linear dependence between these parameters was observed. This could have been due to the fact that not all polyphenols can be considered as antioxidant.

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