Sprouts as potential sources of dietary antioxidants in human nutrition

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ABSTRACT

Purpose: The present study evaluates antioxidant activity, as well as polyphenol and flavonoid contents in common sprouts, available on the Polish market. The aim of this study was to extend our already published food databases.
Materials and methods: Ten seed species from four plant families were analysed. Total polyphenol content of sprout extracts was determined using the Folin-Ciocalteau method. Total flavonoid content was assessed by the aluminium chloride colorimetric method. Total antioxidant status was measured using FRAP and ABTS methods.
Results: The FRAP antioxidant potential was 0.60-2.53 mmol TE (trolox equivalents)/100 g FM (fresh mass), and arranged in descending order it was: white mustard>cress>radish>broccoli>chickpea> sunflower>mung bean>wheat>green lentil>alfalfa), while the ABTS potential was 3.92-16.19 mmol TE/100 g FM (according to decreasing value: white mustard>green lentil>chickpea>sunflower>mung bean>cress> alfalfa>wheat> broccoli> radish). The polyphenol content was 160-774 mg GAE (gallic acid equivalents)/100 g FM, and flavonoid content 15-53 mg QE (quercetin equivalents)/100 g FM.
Conclusion: Our results suggest that sprouts in comparison to other foods, despite small weight can be powerful sources of antioxidants. Special attention in human nutrition should be paid to white mustard sprouts as they are excellent source of polyphenol and flavonoid and are characterized by tremendous antioxidant activity.
Key words: Antioxidant potential, polyphenol, flavonoid, sprouts.

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INTRODUCTION

A growing body of scientific evidence indicates that chronic diseases including cancer, diabetes, cardiovascular, neurological and pulmonary diseases are connected with mechanisms of oxidative and nitrosative stress [1,2]. An excess production of reactive oxygen (ROS) and nitrogen (RNS) species compared to insufficient amounts of protective mechanisms (endogenous: enzymatic and non-enzymatic, and exogenous, such as food with antioxidant properties) leads to imbalance between these pro-oxidants and anti-oxidants [3-5] and as a consequence to inflammation [6] and development of cancer [7]. The initiation of carcinogenesis by these species may result from direct chemical reactions involving oxidation, nitration, halogenation of nuclear DNA, RNA, and lipids or may cause activation of cell signaling pathways [5]. Thus, it is essential to support antioxidant mechanisms with natural nutraceuticals.

The most common group of antioxidants in plant foods are polyphenols, which are divided into five main classes: flavonoids, phenolic acids, stilbenes, lignans, and others. The major and the largest class of polyphenols are flavonoids, among which there are 6 different subclasses: flavan-3-ols, flavonols, flavones, flavanones, anthocyanins, and isoflavones [8]. They exhibit protective effects against a number of oxidative stress-dependent diseases, including cancer [9]. Nonetheless, the ability to absorb and to bioaccumulate naturally occurring antioxidants and chemopreventive substances in human body still remains unknown. There are some concerns about their low water solubility, poor absorption, low bioavailability and insufficient concentration in human body [10], which in consequence do not allow for a systemic or targeted therapy. In order to augment their anticancer activity, some attempts have been made to increase bioavailability of flavonoids in a variety of ways e.g. by self-emulsifying delivery systems, like gels or lipid nanocapsules [11]. On the other side, the antioxidant power of some products has not been establish yet. Therefore, the present study evaluates antioxidant activity, as well as polyphenol and flavonoid contents, in common sprouts having an aim to extend our already published food databases [12-16].

MATERIALS AND METHODS

Sprouts preparation

Ten the most popular in Poland seed species from four plant families: 1. *Asteroideae* family - sunflower (*Helianthus annuus* L.), 2. *Brassicaceae* family - broccoli (*Brassica oleracea* L. *convar. botrytis* (L.) Alef. *var. cymosa* Duch.), cress *(Lepidium sativum* L.*)*, radish (*Raphanus sativus* L.), white mustard (*Sinapis alba* L.), 3. *Fabaceae* family - alfalfa (*Medicago sativa* L.*)*, chickpea (*Cicer arietinum* L.), green lentil (*Lens culinaris* Medik.), mung bean (*Vigna radiata* (L.) Wilczek), 4. *Poaceae* family - wheat (*Triticum aestivum* L.*)* were randomly purchased at different local food markets. The small number of selected seed species was directly related to poor availability of these commercially products on the Polish market, which also indicates limited interest and little knowledge about their health properties.

Before the germination process the seeds were rinsed and soaked separately up to 6 hours in distilled water, according to manufacturer's recommendations. The process was conducted in commercially available tiered clear-plastic sprouter at 20 ± 2 °C and kept out of direct sunlight. Sprouts were rinsed two times a day to provide them with moisture and prevent from souring. After five to six days sprouts were suitable for consumption and harvested.

The sprouts were dried to constant dry matter in an air-drier (MPM, Poland) at 60-70ºC for about 2-3 hours. Dried products were pulverized in a grinder, packed into plastic bags and stored in a desiccator at room temperature until analysis.

Sprouts extraction

Sprouts extraction was based on a method by Saura-Calixto and Goñi [17]. Pulverized samples (0.25 g) were mixed in test tubes with 10 ml of methanol/water (50:50, v/v). The pH was adjusted to 2 using 2M HCl. The tubes were thoroughly shaken at room temperature for 1 h, and then centrifuged at 4000*g* for 10 min. Supernatants were collected in clean dry test tubes. Then, the residues were extracted again with 10 ml of an acetone/water mixture (70/30, v/v). The methanol and acetone extracts were combined and used to determine total polyphenols, flavonoids and antioxidant activity.

Total polyphenol content

Total polyphenol content of sprouts was determined using the Folin-Ciocalteau method [18]. Folin-Ciocalteau reagent consists of two acids ($H_3PW_{12}O_{40}$ and $H_3PMo_{12}O_{40}$). The yellow reagent is oxidized by phenolic compounds occured in sample and creates blue complexes after reduction. In brief, 0.2 ml of an extracted sample was mixed with a 1 ml Folin-Ciocalteau reagent, sample was previously diluted in distilled water (1:10) and 0.8 ml of 7.5 % (w/v) sodium carbonate was added. The absorbance was measured spectro-photometrically after 30 min at 765 nm. Results were obtained from the standard curve and expressed as gallic acid equivalents (GAE) – mg GAE/g dry mass (DM) or 100 g fresh mass (FM).

Total flavonoid content

Total flavonoid content was assessed according to Arvouet-Grand et al. [19], the most common spectrophotometric method based on the formation of aluminium-flavonoid complexes. A 1 ml of 2% aluminium trichloride ($AlCl_3$) diluted in methanol was mixed with equal volume of the extract. Absorption readings were obtained at 415 nm after 10 min against a blank sample consisting of a 1 ml extract solution with 1 ml methanol free of $AlCl_3$. The concentration of total flavonoids in samples was determined from the standard curve and expressed as quercetin equivalents (mg QE/g DM or 100 g FM).

FRAP assay

The FRAP (ferric reducing antioxidant power) was determined according to Benzie and Strain [20]. The principle of this method involves reduction of iron ions from trivalent to divalent form by antioxidants occurred in sample. Divalent iron crates complexes with 2,4,6-tripyridyl-s-triazine (TPTZ) reagent and develop an blue colour reaction. A 1.5 ml of TPTZ solution was warmed to 37°C. Then, a reagent blank was measured at 593 nm. Subsequently, 50 µl of the sample, dissolved in distilled water (1:4), was added to the FRAP reagent. The absorbance was measured following incubation at 37°C for 4 min. The antioxidant potential of samples was determined from the standard curve and expressed as trolox equivalents (mmol TE/g DM or 100 g FM).

ABTS assay

Total antioxidant status was measured using a commercially available TAS kit (Randox Laboratories Ltd, Crumlin, UK). The principle of this method is based on the reduction of ABTS (ABTS – 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) radical cation by antioxidants present in a sample and inhibition of solution darkening. The absorbance was read at 600 nm within 3 min. Antioxidant activity was expressed as trolox equivalents (mmol TE/g DM or 100 g FM).

Statistical analysis

Data analysis was performed using the Statistica 12.0 software (StatSoft, Inc.). The results were expressed as mean ± standard deviation. Correlations between variables were calculated with the Pearson's test. Values of $p<0.05$ were considered statistically significant. All species of sprouts were derived for at least two different producers (N=25) and each were analysed in triplicate.

RESULTS

The characteristics of 10 analysed 6-day sprouts species according to their total polyphenol and flavonoid contents as well as FRAP and ABTS antioxidant activities are shown in Table 1.

Table 1. Characteristics of sprouts in alphabetical order

Species of sprouts	N	Mean dry mass content (%)	polyphenol content		flavonoid content		FRAP activity		ABTS activity	
			mg GAE/ 1 g DM	mg GAE/ 100 g FM	mg QE/ 1 g DM	mg QE/ 100 g FM	mmol TE/ 1 g DM	mmol TE/ 100 g FM	mmol TE/ 1 g DM	mmol TE/ 100 g FM
Alfalfa	3	12	17.0±0.5	213.1±45.6	2.2±0.2	27.0±2.8	0.047±0.01	0.60±0.06	0.63±0.05	7.97±2.05
Broccoli	4	12	24.1±2.4	274.8±49.4	1.4±0.3	15.1±1.6	0.144±0.02	1.62±0.15	0.51±0.15	6.09±2.45
Chickpea	2	38	6.0±0.4	226.7±15.3	0.8±0.0	31.1±1.0	0.034±0.00	1.27±0.13	0.30±0.00	11.41±0.00
Cress	2	10	38.5±1.0	391.2±98.8	2.6±0.1	26.9±7.2	0.218±0.01	2.20±0.40	0.85±0.03	8.71±2.25
Green Lentil	2	27	7.8±0.4	204.1±38.9	1.5±0.1	40.0±4.5	0.026±0.00	0.69±0.11	0.45±0.05	12.02±0.50
Mung bean	3	19	10.5±1.1	199.8±21.2	1.2±0.1	23.0±3.5	0.056±0.00	1.06±0.06	0.47±0.16	8.83±2.34
Radish	3	13	24.7±5.2	294.5±53.5	1.3±0.2	16.6±6.9	0.127±0.03	1.63±0.31	0.41±0.02	3.92±0.89
Sunflower	2	12	21.2±0.4	260.2±3.1	2.4±0.1	29.5±1.0	0.096±0.00	1.18±0.04	0.73±0.00	8.99±0.00
Wheat	2	26	6.7±3.0	159.6±17.5	0.7±0.2	15.3±1.8	0.033±0.00	0.72±0.02	0.32±0.10	7.07±0.81
White mustard	2	19	40.0±0.4	774.0±7.9	2.8±0.2	53.4±3.6	0.131±0.02	2.53±0.40	0.83±0.00	16.19±0.00

N- number of samples, GAE- gallic acid equivalents, QE- quercetin equivalents, TE- trolox equivalents, DM – dry mass, FM – fresh mass

Our study demonstrates that the FRAP antioxidant potential in fresh mass varied 0.6-2.53 mmol TE/100g, and arranged in descending order it was: white mustard>cress>radish> broccoli> chickpea> sunflower>mung bean>wheat>green lentil>alfalfa, while the ABTS potential was 3.92-16.19 mmol TE/100g FM (according to decreasing value: white mustard> green lentil> chickpea> sunflower>mung bean>cress> alfalfa> wheat> broccoli>radish). The polyphenol content in analysed sprouts varied from 160 mg GAE/100 g FM in wheat to 774 mg GAE/100 g FM in white mustard. Similar trends were found for flavonoid content – the highest value was observed in white mustard (53.4 mg QE/100 g FM) and the lowest in wheat (15.3 mg QE/100 g FM) and broccoli (15.1 mg QE/100 g FM). Figure 1 shows that the polyphenol content in fresh sprouts mass positively correlates with flavonoid content (r=0.65, p<0.001), FRAP (r=0.82, p<0.001) and the ABTS antioxidant activity (r=0.56, p=0.004), while the flavonoid content correlates with the ABTS antioxidant activity (r=0.85, p<0.001).

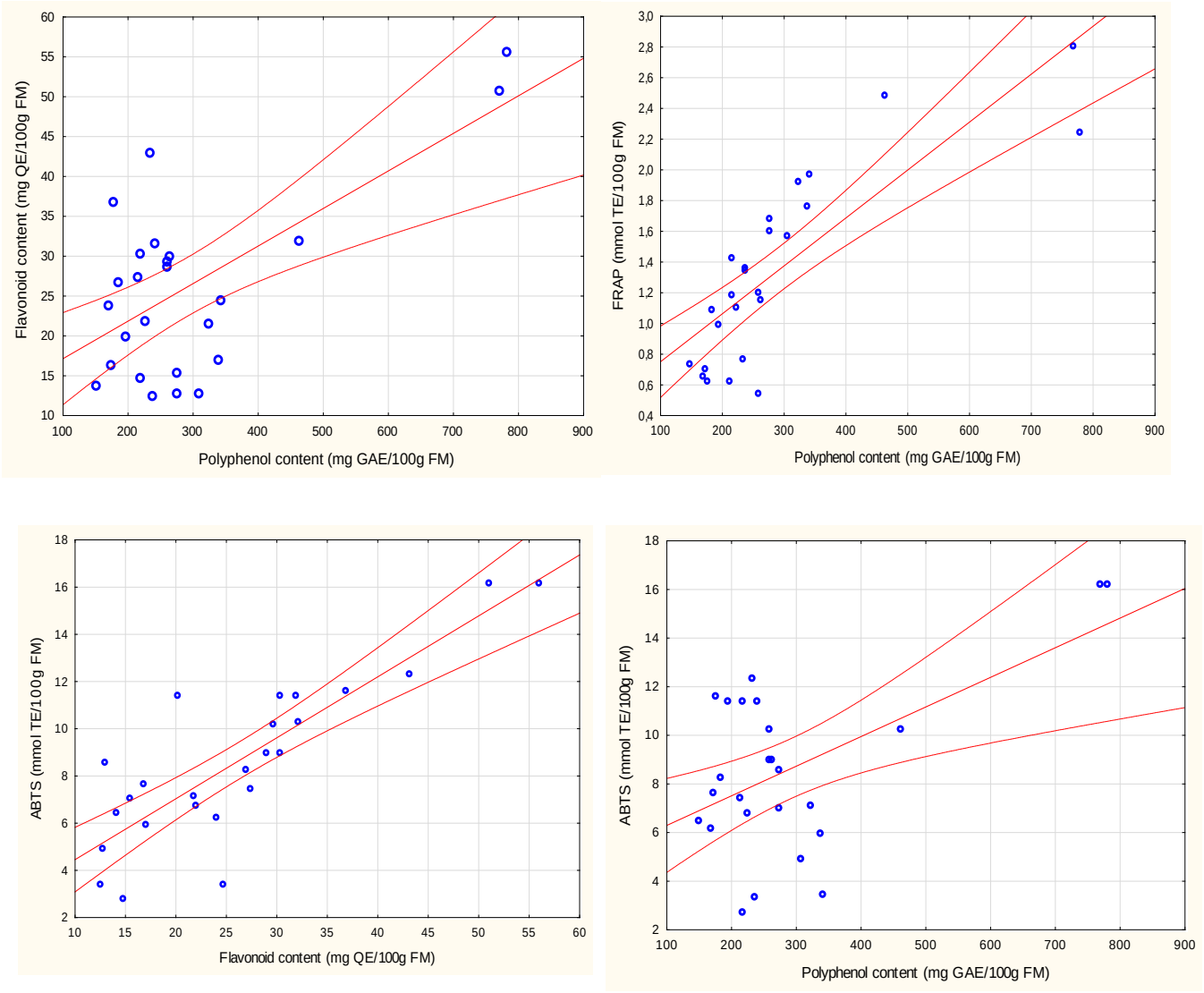

Figure 1. Correlations between polyphenol content and flavonoid content (r=0.65, p<0.001), polyphenol content and FRAP (r=0.82, p<0.001), and ABTS antioxidant activity (r=0.56, p=0.004), and flavonoid content and ABTS antioxidant activity (r=0.85, p<0.001)

DISCUSSION

Earlier study by Soengas *et al.* [21] measured FRAP antioxidant activity in dry mass of Brassica crops (broccoli, cabbage, cauliflower, kale, nabicol and tronchuda cabbage) at four stages after 7, 60, 90, 105 days of sowing. It was found that the FRAP antioxidant activity among all 7-day sprouts varied 0.0041-0.0068 mmol TE/g DM, what was much lower than the results of the own study, which found FRAP antioxidant activity between 0.033 and 0.218 mmol TE/g DM. On the other hand Baenas *et al.* [25] demonstrated that the FRAP antioxidant activity in 8-days nine varieties of Brassicaceae sprouts (broccoli, kohlrabi, red cabbage, rutabaga, turnip, turnip greens, radish, garden cress, and white mustard) ranged 0.45-1.60 mmol TE/100 g FM. The study also found that prolongation of cultivation time caused reduction of antioxidant potential, which in 12-day sprouts was 0.32-1.18 mmol TE/100 g FM. In the own study the FRAP antioxidant capacity in all studied varieties of 6-day sprouts was 0.6-2.53 mmol TE/100 g FM. The collected evidence shows that results may vary between the studies. Generally contents of bioactive compounds in sprouts depend on genotype, environmental stress, growth conditions as well as methods used for their evaluation and extraction [22-26]. Some authors [27] suggested that increasing antioxidant potential in the first stage of plant's growth and gradual decreasing trend in later stage until full maturation, is a consequence of a more active plant metabolism which accompanies rapid growth.

For comparison purposes, the FRAP activities in mmol TE/100 g (ml) FM of selected plant foods are: in vegetables, mushrooms and pulses – 0.03-3.21, fruits – 0.31-2.83, cereal products – 0.06-1.71, beverages – 0.22-2.94, chocolates – 0.55-14.67, nuts and seeds – 0.85-55.91. The total polyphenol content varies for vegetables, mushrooms and pulses: 17-283, for fruits: 72-239, for cereal products: 42-327, for beverages: 30-241, for chocolates: 222-1617, and for nuts and seeds: 125-3529 mg GAE/100 g (ml) FM. Whereas, the total flavonoid content for these food categories are: 2.5-76.2, 5.2-42.3, 2.8-13.6, 2.7-48.3, 8.2-31.3, 8.6-43.2 mg QE/100 g (ml) FM, respectively [12-16]. In comparison to these plant foods assayed with the same procedure as in the current study, sprouts are comparable to most of vegetables, fruits and beverages with respect to FRAP antioxidant potential, polyphenol and flavonoid contents.

Some authors indicate, that sprouts contain significantly more concentrated nutrients, such as vitamin C, B vitamins and polyphenols, as well as they exhibit higher antioxidant potential, than the seeds or mature vegetables [28,29]. They are an excellent example of "functional foods", which can reduce the risk of developing of various diseases due to the content of bioactive components. Nowadays, when commonly used synthetic food additives are reported to induce DNA damage, more attention is focused on natural plant foods.

In the current study, antioxidant potential and polyphenol content were the highest in the white mustard sprouts. According to the study carried out by Zielniok *et al*. in white mustard seeds in the largest quantity are present such polyphenols as: catechins (especially catechin and epicatechin) and simple phenolic acids (i.e. ellagic acid, hydroxybenzoic acids and ferulic acid) [30]. These authors have demonstrated, that white mustard polyphenols with high antioxidant activity may have beneficial effects on the muscle tissue (C2C12 mouse skeletal muscle cells) which is characterized with the intensive metabolism, by increasing the concentration of reduced glutathione and upregulating glutathione reductase and peroxidase activity. Moreover, white mustard polyphenols are able to decrease the level of oxysterols, showing the protective effect on a cell membrane.

The polyphenol content in fresh sprout mass in the current study positively correlated with the antioxidant potential (FRAP, ABTS), what is in agreement with the own previously published studies and the others [12,13,17]. Several analytical methods can be used to measure the total antioxidant capacity of food. They vary in the mechanism of generation of different radical species and the detection methods of the end-products of reaction. In the current study we selected two colometric methods: the FRAP method and the ABTS method , which are usually used to measure the antioxidant potential of foods. According to Arts *et al.* [31] ABTS assay measures the antioxidant capacity of the parent compounds plus that of the reaction products, thus the results may be higher in relation to the FRAP method which explains the differences obtained between the methods used and the descending order of antioxidant potential of each seed species. FRAP method is simple, economic, and the results are repeatable, therefore this method was applied to measure the antioxidant potential of the large group of food products, published previously [32].

CONCLUSION

To sum up, the results suggest that sprouts, especially white mustard sprouts, can be valuable sources of antioxidants, therein polyphenols and flavonoids, and should occupy a prominent place in human nutrition.

Conflicts of interest

None declared

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