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Sprouts of selected plants as a source of bioavailable antioxidants and lipoxygenase inhibitors

Kiełki wybranych roślin jako źródło biodostępnych przeciwutleniaczy oraz inhibitorów lipoksygenazy

INTRODUCTION

The pathology of numerous chronic diseases, including cancer and heart disease, involved oxidative damage to cellular components. Phenols have been widely studied and their beneficial influence on human health was confirmed. The beneficial effect comes in part through the antioxidant characteristics of phenols; therefore, it is important to evaluate their antioxidant activities. Due to the potential significance of phenolic antioxidants for the prevention of a wide range of degenerative physiological processes, it is necessary to identify plant sources with optimum physiological stages for maximizing phenolic accumulation [3].

One of the principal topics concerning the beneficial effects of polyphenols is their bioavailability and metabolic purpose. Bioavailability is the proportion of the nutrient that is digested, absorbed and metabolized through normal pathways [4]. The gastro-intestinal tract may be considered as an extractor where both the mechanical action during mastication and the chemical action during the digestive phase contribute to the extraction of phenolic compounds [9]. It has been widely reported that seed sprouts provide higher nutritive value than raw seeds and their production is simple and inexpensive. Thus, the aim of this study was to evaluate of bioaccessibility and bioavailability of biologically active compounds from commonly consumed sprouts.

MATERIAL AND METHODS

Dry seeds of cress, radish, alfalfa and lentil were purchased in a local garden shop. Seeds were sterilized with 1% sodium hypochloride for 5 min. Sodium hypochloride was removed by washing with sterile water. After disinfection, seeds were allowed to absorb water at 25°C for 6 h. Seeds were dark germinated for 6 days in sterile Petri plates with humidified Whatman Grade No. 2 Filter Paper at 25°C. Three kinds of extracts were then prepared:

1) buffer extract (BE): 0.5 g of fresh sprouts was mixed with 4 ml of PBS and extracted by shaking at 37°C for 60 min. After centrifugation (15 min, 20°C, 3000 g) supernatants were recovered and the extraction procedure was repeated. After centrifugation (15min, 25°C, 3000*g*) supernatants were combined and used in the further studies.

2) digestion extract (DE): 2g of fresh sprouts were subjected to simulated gastrointestinal digestion according to Elles et al. [5].

3) absorption extract (AE): 10 ml of the DE was transferred into the dialysis sacks (D9777-100FT, Sigma-Aldrich), placed in an tube containing 50 ml of PBS and incubated in a rotary shaker (2x 2h, 37°C). The PBS buffer together with the compounds that passed thought the membrane (dialysate) were treated as an equivalent of the raw material absorbed in the intestine after digestion.

The amount of total phenolics was determined using Folin-Ciocalteau reagent and expressed as gallic acid equivalent (GAE) mg/g f.w. [11]. Total flavonoids content was determined according to the method described by Bahorun et al. [2] and expressed as quercetin equivalent (QE) mg/g f.w. Phenolic acids content was determined according Szaufer-Hajdrych [12] and expressed as caffeic acid equivalent (CAE) μg/g f.w. Lipoxygenase inhibitors activity was determined spectrophotometrically according to the method described by Axelroad et al. [1]. The free radical scavenging activity was measured according to Re et al. [10]. Chelating power was determined by the method of Guo et al. [7]. Reducing power was determined by the method of Oyaizu [8].

Bioaccessibility and bioavailability of bioactive compounds were calculated according to following equations:

- phenolics bioaccessibility factor (BAC_P):

BAC_P [%] = C_D/C_R x 100

where: C_D – concentration of compounds after simulated gastrointestinal digestion, C_R - concentration of compounds after PBS extraction (raw extract),

- phenolics bioavailability index (BAV_P):

BAV_P [%] = C_A/C_D x 100

where: C_A – concentration of compounds after simulated intestinal absorption, C_D - concentration of compounds after simulated gastrointestinal digestion,

- the antioxidant bioaccessibility index (BAC):

BAC [%]= [1-(A_D/A_R)] x 100

where: A_D –IC_{50} of extract after simulated gastrointestinal digestion, A_R - IC_{50} of raw extract (after PBS extraction)

- the antioxidant bioavailability index (BAV):

BAV[%]= [1-(A_A/A_D)] x 100

where: A_A - IC_{50} of extract after simulated intestinal absorption, A_D – IC_{50} of extract after simulated gastrointestinal digestion,

All experimental results were mean ± S.D. of three parallel measurements and data were evaluated by using one-way analysis of variance (Tukey test). P values < 0.05 were regarded as significant.

RESULTS

As Table 1 presents, all sprouts contained significant amounts of phenolic compounds (including flavonoids and phenolic acids).Taking into account total phenolics and flavonoids content, the analyzed sprouts were ordered as follows: cress>radish >alfalfa > lentil. It is worth nothing that simulated digestion released significant amounts of polyphenolics from food matrix. As Table 2presents, all sprouts were a good source of bioaccessible phenolics, especially flavonoids. Unfortunately, their bioavailability was relatively low in all cases with the exception of lentil sprouts. Generally, lentil was the best source of bioaccessible and bioavailable phenolic compounds. Lentil sprouts were the best source of bioavailable phenolics (determined with Folin reagent) and flavonoids (BAV_p 100.10% and 116.65%, respectively). Bioavailability of phenolics from other sprouts did not exceed 50%, whereas flavonoids bioavailability ranged from 16.85% to 36.15%. The best source of bioavailable phenolic acids were radish sprouts (Tab. 2.). Tested sprouts were possessed of relatively high amount of free radical scavengers.

It should be noted that simulated digestion caused a decrease of studied activity (Fig. 1A.).

Table 1. Total phenolics, total phenolic acids and flavonoids content in sprouts samples. BE – buffer extract, DE-extract after digestion *in vitro*, AE-extract after absorption *in vitro*

Sprouts	Sample	Total phenolics content [mg/g f.w.]	Total phenolic acids content [μg/g f.w.]	Total flavonoids content [mg/g f.w.]
Alfalfa	BE	107.31 ± 5.26 ab	12.47 ± 1.25 f	0.53 ± 0.05 ad
	DE	176.41 ± 6.45 f	15.14 ± 0.98 d	11.59 ± 0.78 f
	AE	88.08 ± 2.45 c	5.19 ± 0.75 a	4.19 ± 0.22 c
Cress	BE	210.95 ± 5.98 g	16.78 ± 0.78 d	1.08 ± 0.02 d
	DE	281.56 ± 9.56 d	31.64 ± 2.99 c	17.92 ± 0.89 g
	AE	116.91 ± 2.54 ab	4.38 ± 1.21 ab	5.44 ± 0.06 e
Radish	BE	136.92 ± 7.11 b	29.49 ± 1.56 c	0.44 ± 0.03 a
	DE	275.87 ± 11.24 d	29.62 ± 2.01 c	3.90 ± 0.12 bc
	AE	122.82 ± 5.98 ab	4.99 ± 0.25 a	6.12 ± 0.05 e
Lentil	BE	65.98 ± 3.33 e	4.41 ± 0.33 ab	0.34 ± 0.07 a
	DE	106.44 ± 3.11 ac	7.92 ± .041 e	3.02 ± 0.11 b
	AE	106.55 ± 7.41 ac	3.16 ± 0.22 b	3.52 ± 0.14 bc

*a,b,c,d,e,f,g – the same letters in columns represent statistically not significant differences ($p<0.05$)

Table 2. Bioaccessibility (BAC_p) and bioavailability (BAV_p) of phenolic compounds from selected sprouts

Factor	Compounds	Lentil	Radish	Cress	Alfalfa
BACP [%]	Total phenolics	161.32 a	201.48 c	133.47 b	164.39 a
	Flavonoids	888.24 a	886.36 a	1659.26 b	2186.79 c
	Phenolic acids	179.59 a	100.44 b	188.56 a	121.41 c
BAVP [%]	Total phenolics	100.10 c	44.52ab	41.52a	49.93b
	Flavonoids	116.56 c	16.85 b	30.36 a	36.15 a
	Phenolic acids	39.90 c	156.92 d	13.84 a	34.28 b

*a,b,c – the same letters in columns represent statistically not significant differences ($p<0.05$)

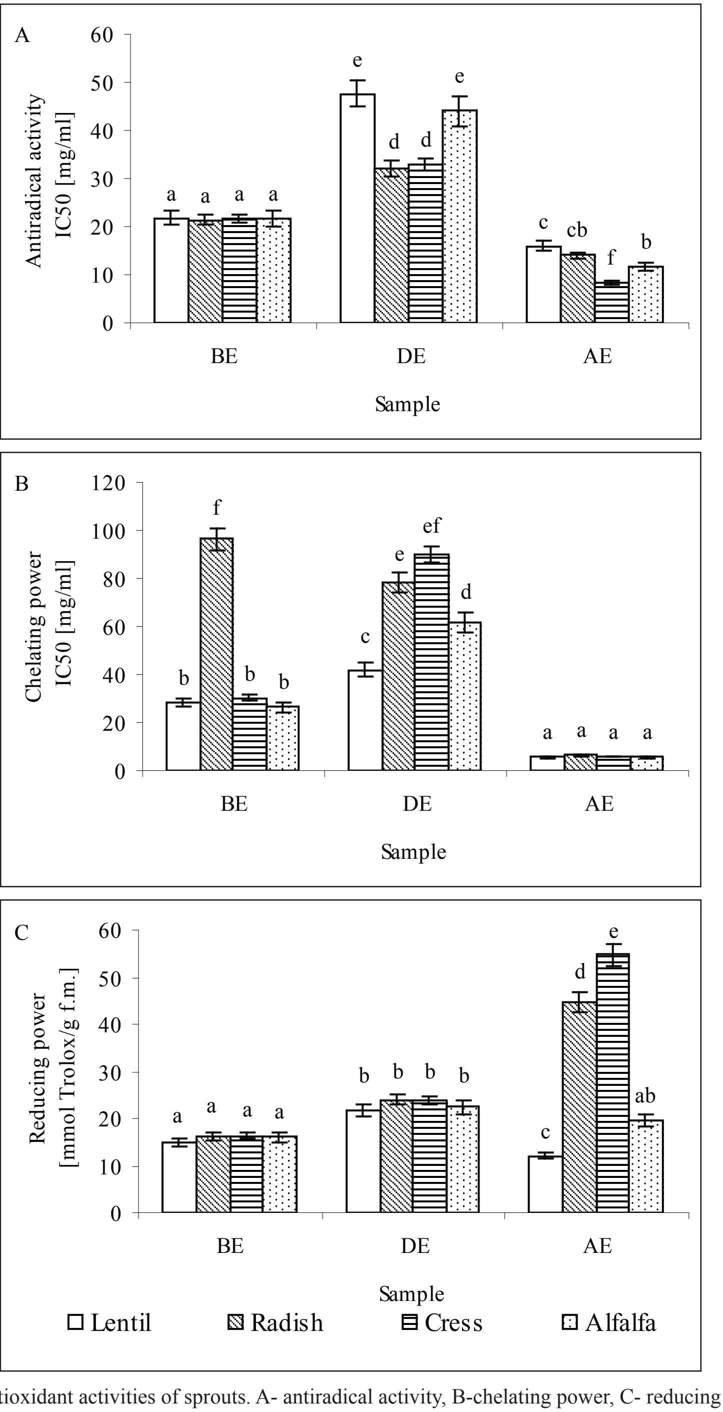

Fig.1. Antioxidant activities of sprouts. A- antiradical activity, B-chelating power, C- reducing power.
*a,b,c,d,e,f – the same letters represent statistically not significant differences (p<0.05)

Bioavailability of free radical scavengers was relatively high and ranged from 56.29% in radish to 73.66% in alfalfa sprouts (Tab. 4.). Raw and digested samples showed relatively low chelating activity (Fig. 1B.). On the other hand metal ion chelators have been suggested as potential the most bioavailable antioxidants (BAV about 90%) (Tab. 4). Among the studied sprouts the highest reducing power was possessed by absorbed extracts of radish and cress, 44.73 and 54. 34 µmolTrolox/ g f.m., respectively (Fig.1C). All tested sprouts contained bioavailable reductive compounds – the best source of them were Brasicaceae sprouts (BAV= 186.21% for radish and 230.21% for cress). The highest inhibitory activity against LOX was found for radish, cress and alfalfa sprouts. Digestion on simulated gastrointestinal condition caused a release of bioactive compound with LOX inhibitory activity (Tab. 3). All sprouts contained bioaccessible LOX inhibitors, lentil sprouts were the best source of them (BAC=81.56%). The studied sprouts, except alfalfa (BAV about -210.49%) contained bioavailable LOX inhibitors, bioavailability ranged from 60.8% to 72.03% (Tab. 4).

Table 3. Inhibition of lipoxygenase expressed as IC_{50} (mg f.w./ ml)

Plant	Sample		
	BE	DE	AE
Lentil	339.81± 21,43 c	62.68 ± 4,56 a	20.18 ± 1,56 b
Radish	74.42 ± 5,12 a	58.00 ± 1,56 a	16.22 ± 0,99 a
Cress	60.03 ± 2,61 b	38.43 ± 0,98 b	15.06 ± 0,87 a
Alfalfa	74.84 ± 3,56 a	46.68 ± 2,02 c	144.93 ± 5,87 c

*a,b,c – the same letters in columns represent statistically not significant differences ($p<0.05$)

Table 4. Bioaccessibility (BAC) and bioavailability (BAV) of phenolic and bioactive compounds from selected sprouts

Factor	Compounds	Lentil	Radish	Cress	Alfalfa
BAC [%]	ABTS scavengers	-118.42	-49.19	-52.00	-103.84
	Metal ions chelators	-50.29	18.53	-197.75	-134.17
	Reductors	81.21	275.25	336.78	121.71
	LOX inhibitors	81.56	22.06	35.99	37.63
BAV [%]	ABTS scavengers	66.38	56.29	74.76	73.66
	Metal ions chelators	86.74	91.88	93.32	90.98
	Reductors	56.17	186.21	230.21	86.87
	LOX inhibitors	67.81	72.03	60.80	-210.49

DISCUSSION

From the moment the seed breaks dormancy, protective responses emerge through the synthesis of phenolics and other compounds. Phenols have been confirmed to possess diverse bioactivities which could be beneficial to human health [3]. In our study the best source of phenolic compounds were Brassicaceae (cress and radish) sprouts. These results confirmed those obtained by Cevallos-Casals and Cisneros-Zevallos [3]. One of the principal topics concerning the beneficial effects of polyphenols is their bioavailability. As Table 2 presents all sprouts were a good source of bioaccessible phenolics, especially flavonoids, but their bioavailability was relatively low (in all cases with the exception of lentil sprouts). Similar results were obtained by Tarko et al. [14] in the study concerning bioavailable compounds from apples, plums, pear and banana and by Gil-Izquierdo et al. [6] in the study concerning changes of flavanones from different orange juices. Previous publications detailing the changes of phenolic compounds content and changes of antioxidant activity after in vitro digestion and absorption of vegetables are sparse. Results obtained in our study showed that digestion in simulated gastrointestinal conditions caused decrease of antiradical activity and chelating power in all samples except chelating power in radish sprouts. It is well known from the literature that the radical scavenger activity of polyphenols is strongly pH-dependent [13]. In our study bioavailability of free radical scavengers and metal chelators were relatively high, whereas only lentil and alfalfa sprouts contained bioavailable reductive compounds. Tarko et al. [14] showed that dialysates of apples and plum showed greater free radical scavenging capacity in the comparison to fruits before digestion.

Creating reactive oxygen species (ROS) is bound, inter alia, with lipoxygenase (LOX) activity. Digestion in vitro caused a release of LOX inhibitors from all tested sprouts. The studied sprouts, except alfalfa contained bioavailable LOX inhibitors. In recent literature there is lack of information concerning bioaccessibility and bioavailability of LOX-inhibitors from sprouts. Since extracts show high antioxidant and LOX inhibitory power, it is possible that these activities may be involved in their putative medicinal properties.

CONCLUSION

The tested sprouts were confirmed as a good source of bioaccessible and bioavailable phenolic compounds. The best bioavailability of them was obtained for lentil sprouts case. All sprouts possessed bioavailable compounds with chelating and antiradical activity and, with exception of alfalfa sprouts, effective LOX inhibitors. This results confirmed the fact that plant seedlings contained compounds with multi-directorial health-promoting activities.

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SUMMARY

The beneficial effect of phenolics comes in part due to their antioxidant activity. Sprouts provide higher nutritive value than raw seeds. Thus, the aim of this study was to evaluate bioaccessibility and bioavailability of biologically active compounds from commonly consumed sprouts. Lentil sprouts were the best source of bioavailable phenolics and flavonoids (about 100%). Bioavailability of phenolics from other sprouts did not exceed 50%, whereas flavonoids bioavailability ranged from 16.85% to 36.15%. Bioavailability of free radical scavengers ranged from 56.29% in radish to 73.66% in alfalfa sprouts. Metal ion chelators were potentially the most bioavailable antioxidants (about 90% in all cases). All tested sprouts contained bioavailable reductive compounds, whereas the best source of them were Brasicaceae sprouts (BAV= 186.21% for radish and 230.21% for cress). Studied seedlings, except alfalfa, contained bioavailable lipoxygenase inhibitors, bioavailability ranged from 60.8% to 72.03%. Results confirmed the fact that plant seedlings contained compounds with multi-directorial health-promoting activities.

Keywords: phenolic compounds, sprouts, bioaccessibility, bioavailability, lipoxygenase inhibition

STRESZCZENIE

Prozdrowotne działanie związków fenolowych związane jest z ich aktywnością antyoksydacyjną. Kiełki charakteryzują się wyższą wartością odżywczą niż suche nasiona, stąd też celem pracy było określenie biodostępności i bioprzyswajalności aktywnych biologicznie związków występujących w powszechnie konsumowanych kiełkach. Kiełki soczewicy były najlepszym źródłem bioprzyswajalnych związków fenolowych ogółem i flawonoidów (około 90%). Bioprzyswajalność związków fenolowych z pozostałych kiełków nie przekraczała 50%, podczas gdy biodostępność flawonoidów wynosiła od 16,85% do 36,15%. Bioprzyswajalność związków przeciwrodnikowych zawierała się w przedziale od 56,29% (rzodkiewka) do 73,66% (lucerna). Związki zdolne do chelatowania jonów metali były wysoce bioprzyswajalne (około 90% we wszystkich przypadkach). Wszystkie badane kiełki zawierały bioprzyswajalne związki redukujące, najlepszym ich źródłem były kiełki roślin kapustowatych (186,21% w przypadku rzodkiewki i 230,21% w przypadku rzeżuchy). Badane kiełki, oprócz kiełków lucerny zawierały bioprzyswajalne inhibitory lipoksygenazy, ich bioprzyswajalność wynosiła od 60,8% do 72,03%. Otrzymane wyniki potwierdzają tezę, iż kiełki zawierają związki o wielokierunkowym działaniu prozdrowotnym.

Słowa kluczowe: związki fenolowe, kiełki, biodostępność, bioprzyswajalność, inhibicja lipoksygenazy.