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Impact of germination on phenolic content and antioxidant activity of 13 edible seed species

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ABSTRACT

The aim of this work was to test 13 edible seeds for the levels of phenolic compounds and the antioxidant activity (TAC) at different germination states (dormant, imbibed and 7d sprouts). Selected seeds included mungbean, alfalfa, fava, fenugreek, mustard, wheat, broccoli, sunflower, soybean, radish, kale, lentil and onion. Accumulated phenolics (mg chlorogenic acid equivalent, CAE) and TAC (μg Trolox equivalent) on dry basis (DB) showed the general trend distribution of 7d sprouts > dormant seeds > imbibed seeds. In addition, the specific TAC (μg Trolox mg^{-1} CAE) increased only for imbibed seeds indicating a possible protection effect of the phenolic antioxidants to the emerging sprouts. Phenolic contents of 7d sprouts (DB) ranged from 490 (lentil) to 5676 (mustard) mg CAE 100 g^{-1}. Seven day sunflower sprouts had higher TAC on a DB (40202 μg Trolox g^{-1}) compared to other seeds (1456–25991) and a blueberry reference (35232). Increases in phenolics (DB) from dormant seed to 7d sprout differ among seeds, ranging from 2010% (mungbean) to −11% (kale), while increases in TAC (DB) ranged from 1928% (mungbean) to 0% (lentil). This study shows that germinated edible seeds are an excellent source of dietary phenolic antioxidants.

1. Introduction

Phenols have been widely studied and confirmed to possess diverse bioactivities which could be beneficial to human health. They have been related to reduce the risks of cancer, heart disease, and diabetes; inhibition of plasma platelet aggregation, cyclooxygenase (COX) activity, and histamine release; as well as to *in vitro* antibacterial, antiviral, anti-inflammatory, and anti-allergenic activities (Oak, El Bedoui, & Schini-Kerth, 2005; Shetty, 2004; Yang, Landau, Huang, & Newmark, 2001; Yao et al., 2004). The benefits towards many of these conditions come in part through the antioxidant characteristic of phenols; therefore, it is important to quantify, identify and 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 maximising phenolic accumulation. Little is known about variations in phenolic concentrations during seed germination.

Germination starts when the dry seed begins to take up water and is completed when the embryonic axis elongates. At this point reserves within the storage tissues of the seed are mobilised to support seedling growth. (Bewley, Hempel, McCormick, & Zambryski, 2001). From the moment the seed breaks dormancy, protective responses emerge through the synthesis of phenolics and other compounds (Taiz & Zeiger, 1998). It is not clear how the level of phenolics, especially phenolic antioxidants, vary throughout seed germination. We hypothesised that phenolic synthesis and their antioxidant activity will change with germination stage. Changes in phenolic synthesis and antioxidant activity would indicate seed preparation towards adverse conditions. Identifying germination stage where the level of phenolic antioxidants is optimised would be attractive for the growth of edible sprouts with enhanced nutraceutical properties. In the present study, our objective was to characterise the levels of phenolic compounds and antioxidant properties of 13 selected seed species at different germination stages, including dormancy, imbibition and sprouting and to correlate them with the nutraceutical value of seeds.

2. Materials and methods

2.1. Materials

Fava bean (*Vicia faba*), sunflower (*Helianthus annuus*), green lentil (*Lens esculenta*), onion (*Allium cepa*), mung bean (*Vigna radiata* L. Wilczek), mustard (*Brassica juncea*), radish (*Raphanus sativus* 'Daikon'), wheat (*Triticum aestivum*), alfalfa (*Medicago sativa*), kale

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(*Brassica napus pabularia* 'Red Russian'), fenugreek (*Trigonella foenum-graecum*) and soybean (*Glycine max* 'Butterbeans') seeds were purchased from Johnny's Selected Seeds (Winslow, ME, USA), while broccoli (*Brassica oleracea* var. Italica 'Decicco') seeds from Holmes Seeds (Canton, OH).

Chlorogenic acid, trolox (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium carbonate and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Seed germination

Seeds were sterilised with 70% ethanol for 2.5 min, followed by 2.5% sodium hypochlorite for 15 min (Huang, Haig, Wu, An, & Pratley, 2003). Ethanol and sodium hypochlorite were removed with four rinses of sterile water. After disinfection, seeds were allowed to imbibe water at 18 °C for 17 h. Then water was removed and seeds were dark-germinated in sterile petri plates with humidified Whatman no. 2 filter papers at 18 °C. Filter paper was kept moist by spraying with sterile water as needed.

Seeds were assayed for dry matter, total phenolics and total antiradical capacity (TAC) through time. Depending on their size and weight, 3 (fava), 8 (soybean), 10 (mungbean, sunflower), 15 (lentil), 20 (fenugreek), 25 (radish), 30 (kale, wheat), 40 (broccoli, onion), or 70 (alfalfa, mustard) seeds represented one replicate. Three to six replicates were conducted for each assay.

2.3. Total soluble phenolics

Total soluble phenolic content of methanolic extracts was assayed as described by Cevallos-Casals and Cisneros-Zevallos (2003) using Folin–Ciocalteu reagent with final reaction measurements conducted at 725 nm. Phenolics were not determined in onion seeds due to a precipitate formed during the assay. Total phenolics were expressed as mg chlorogenic acid equivalents (CAE) 100 g^{-1} wet basis (WB), dry basis (DB) or per seed basis (PSB), based on a standard curve.

2.4. Total antiradical capacity (TAC)

TAC of phenolic compounds was adapted from Brand-Williams, Cuvelier, and Berset (1995). The same methanol extract as for phenolics was used. A total of 150 μl of sample (equivalent methanol volume to control) reacted with 2850 μl DPPH (98.9 μM in methanol) in a shaker covered with aluminum foil at 20 °C. Readings at 515 nm were taken after 20 h reaction time. The change in absorbance was used and results were expressed as μg Trolox equivalents g^{-1} WB, DB or PSB, from a standard curve. In addition, specific antioxidant capacity (specific TAC) was defined as the ratio of total antiradical capacity/total soluble phenolics and expressed as μg Trolox equivalents mg^{-1} CAE. The specific antioxidant capacity provides information on the effectiveness of phenolics to neutralise free radicals. A higher specific TAC means phenolic compounds have a higher capacity to stabilise free radicals.

2.5. Isolation of phenolic compounds with C-18 resin

For confirming that phenolic compounds in methanol extracts were the major compounds reacting with DPPH and Folin–Ciocalteu reagents, phenolic compounds from representative seeds were isolated with C-18 cartridges and reacted with DPPH and Folin–Ciocalteu. Methanol extracts were concentrated to dryness on a Speed Vac Concentrator (Model SV0–100H, Savant Instruments, Inc., Hicksville, NY) at 35 °C attached to an aspirator pump. Samples were re-diluted with acidified (0.01% HCl) water. Aqueous samples were applied to Sep-Pak Plus C-18 cartridges (Waters Assoc., Milford, MA), previously activated with acidified methanol followed by acidified water. Water-soluble compounds, including sugars and acids, were eluted with acidified water and phenolics were recovered with acidified methanol.

2.6. Analysis of variance and covariance

One-way analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were performed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Means were compared with Duncan's Multiple Range Test at $\alpha = 0.01$ or 0.05.

3. Results and discussion

3.1. Changes in total phenolics and antioxidant capacity of seeds at different germination stages

The values of total phenolics and TAC for a dry or dormant seed would indicate the amount of phenolic antioxidants synthesised while the seed was attached to the parent plant, while for imbibed seed and 7d sprout the total phenolics and TAC values indicate synthesis of phenolic antioxidants after dormancy (Figs. 1 and 2).

Concentrations expressed on a WB may be influenced by changes in moisture content presenting a dilution effect on the synthesis of phenolics. When results are expressed on a DB, the moisture component is eliminated. On PSB a potential understanding of total yields per seed unit was obtained; therefore results in DB and/or PSB was preferred.

In the dormant stage, phenolic content and TAC values on a DB for dormant seeds represented an average contribution of ~28.5% (5–85% range) and ~30.7% (9–100% range), respectively, compared to sprouts after a 7d germination process, indicating that most of the synthesis of phenolics occurs during imbibition and seed growth (Figs. 1B, 2B and 3). Phenolic content on a WB for dormant seeds was higher than that for imbibed seeds and 7d sprouts (except for fava and mungbean), suggesting a dilution effect of phenolics after water imbibition and growth due to an increase in water absorption (Fig. 1C, Table 1).

The imbibition stage showed to be an active period of phenolic antioxidant synthesis for most seeds. Of the total phenolics and TAC accumulated within 7 days of germination, synthesis during seed imbibition (17 h) accounted for ~11% (0–25% range) and ~25.4% (0–43% range), respectively (Figs. 1B and 2B). Wheat and lentils were the only seeds not experiencing an increase in both total phenolics and TAC during imbibition while soybean did not show an increase in phenolics.

The specific TAC (normalised to phenolic content) was usually higher for imbibed seeds as compared to dormant seeds, especially in alfalfa and onion (Fig. 2C). This indicates that phenolic compounds with a higher number of DPPH reactive hydroxyl groups have been synthesised during water imbibition. This was further confirmed by plotting phenolic content against TAC for all seeds, except sunflower (Fig. 4). Results showed that the slope of the linear regression fit for imbibed seeds was statistically higher ($\alpha = 0.01$ ANCOVA) than that for dormant seeds, indicating that at similar phenolic contents, TAC will be higher for imbibed seeds. A higher specific TAC for imbibed seeds could suggest that the first steps the seed response machinery takes after breaking dormancy is to synthesise phenolic compounds with higher than normal antioxidant activity so as to protect hypocotyl growth against oxidative reactions triggered by environmental factors.

For most seeds during the sprout growth stage, ~59.7% (0–87% range) of the total phenolics found in 7d sprouts were synthesised after imbibition and these synthesised phenolics accounted for ~43.8% (0–88% range) of the final TAC (Figs. 1B, 2B and 3). Exceptions

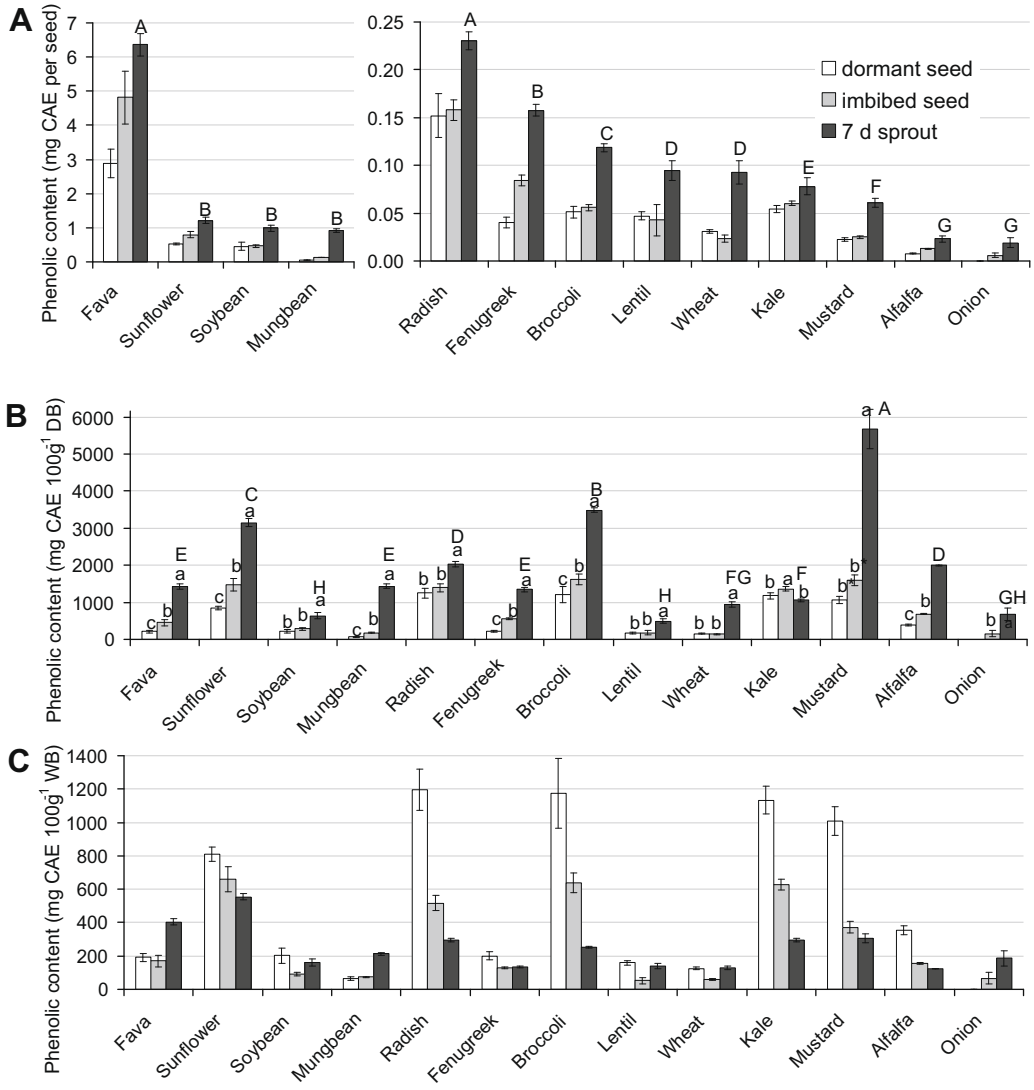

Fig. 1. Phenolic content of seeds of 13 plant species at three different germination stages grown at 18 °C. A = per seed basis (PSB), B = dry basis (DB), C = wet basis (WB). Phenolic content was expressed in mg chlorogenic acid equivalents. 7d sprouts with similar upper case letters within each figure are not significantly different ($\alpha = 0.05$ with Duncan test) from each other. Seed stages with the same lower case letter are not significantly different ($\alpha = 0.05$ with Duncan test) from each other within the same plant species. *Dormant and imbibed mustard seed were significantly different ($\alpha = 0.05$ with Duncan test) when ran independent of 7d sprout. Total phenolic values for dry onion seeds could not be obtained. Bars show the average of 3–6 replicates ± standard deviation.

were kale seeds which did not show an increase in phenolics while lentils and onion did not show an increase in TAC during this stage.

The increases in phenolic content on a DB from dormant seed to 7d sprout were in the order mungbean (2010%) > fava (586%) > wheat (535%) > fenugreek (530%) > mustard (435%) > alfalfa (409%) > sunflower (271%) > soybean (201%) > broccoli (186%) > lentil (185%) > radish (63%) > kale (−11%) (Fig. 1B). These results show mungbean to be the seed with the greatest increase in phenolics since ~95% of the total phenolics were synthesised after imbibition and during 7d growth (Fig. 3). For TAC on a DB, the increases were in the order mungbean (1928%) > alfalfa (943%) > fava (919%) > fenugreek (681%) > mustard (566%) > wheat (433%) > broccoli (290%) > sunflower (261%) > soybean (175%) > radish (117%) > kale (16%) > lentil (0%) (Fig. 2B). These increases in phenolic content and TAC show potentially important roles of phenolics during seed germination, as well as the potential enhancement of the nutraceutical value of seeds by the germination process.

After 7 day growth, phenolics on average, had a lower reaction efficiency against DPPH radicals [$y = 5.267x$, $R^2 = 0.807$ (data not shown)], as compared to imbibed seeds [$y = 7.1249x$, $R^2 = 0.9492$ (Fig. 4)]. This could be caused by the oxidation of antioxidant phenolics and their utilisation as precursors of lignin or lignan structures (de Ascensao & Dubery, 2003). It is also possible that the synthesis rate of phenolics with high antioxidant activity decreased or stopped and the synthesis rate of phenolics with lower number of DPPH reactive OH groups, increased.

When the phenolic compounds of sunflower, fava, radish and mungbean at dormant, imbibed and 7d sprout stage were isolated using C-18 cartridges, no significant difference (p-value > 0.05) was found between the phenolic specific TAC of C-18-purified phenolic compounds as compared to the phenolic specific TAC of methanolic extracts. These results confirm that phenolic compounds are responsible for most of the antioxidant properties of the sample methanolic extracts, thus reinforcing the validity of expressing TAC on a phenolic basis.

In general, accumulated phenolic content and TAC on a DB for most seeds showed a consistent trend, where 7d sprouts > imbibed seeds. The phenolics synthesised throughout the germination process could serve as protection against environmental factors and for structure-giving.

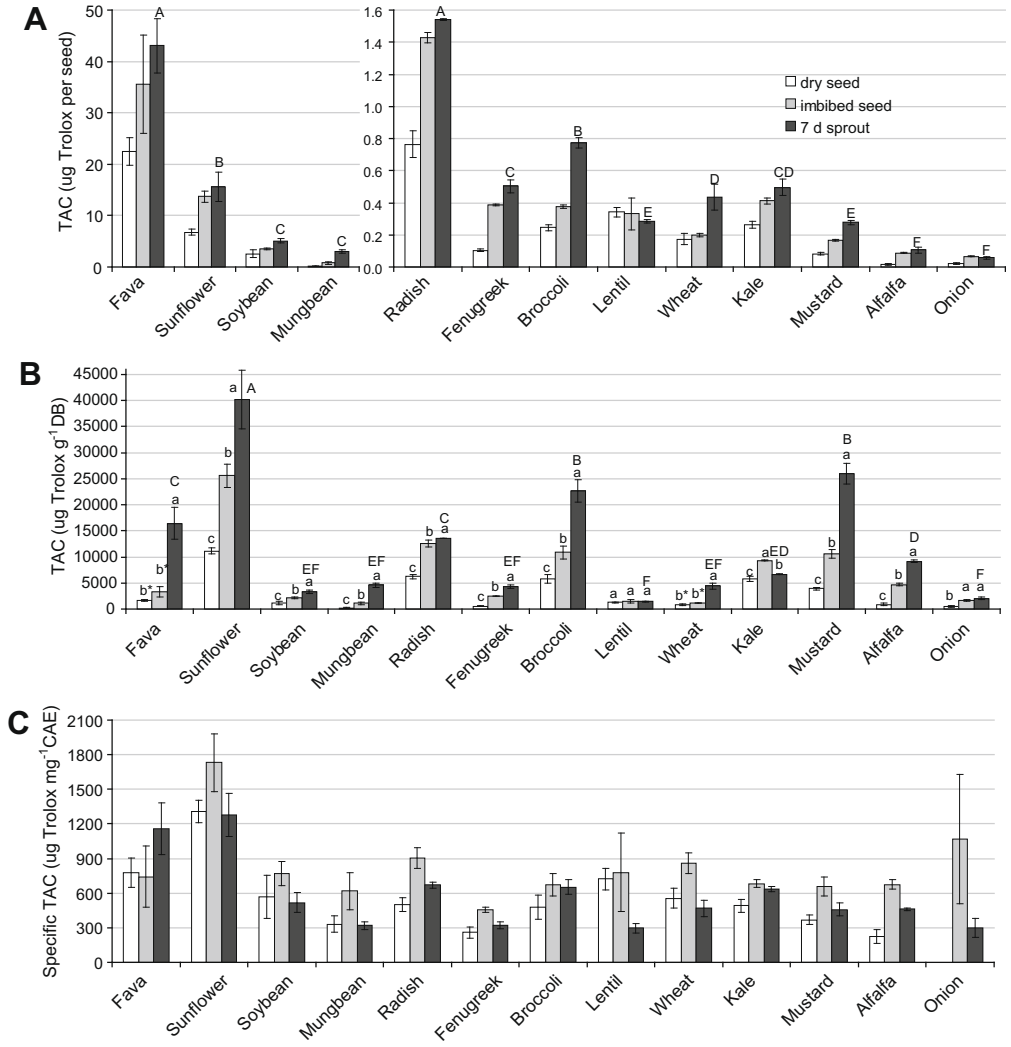

Fig. 2. TAC of seeds of 13 plant species at three different germination stages grown at 18 °C. A = per seed basis (PSB), B = dry basis (DB), C = TAC based on phenolic basis. TAC was expressed in μg Trolox equivalents. 7d sprouts with similar upper case letters within each figure are not significantly different ($\alpha = 0.05$ with Duncan test) from each other. Seed stages with the same lower case letter are not significantly different ($\alpha = 0.05$ with Duncan test) from each other within the same seed. *Dormant and imbibed stages for fava and wheat seeds were significantly different ($\alpha = 0.05$ with Duncan test) when ran independent of 7d sprout stage. Specific TAC values for dry onion seeds could not be obtained. Bars show the average of 3–6 replicates ± standard deviation.

3.2. Nutraceutical value of selected seed species

Sunflower seeds showed high phenolic content (on DB and WB) and both the highest TAC on a DB and specific TAC (Figs. 1 and 2). Imbibed sunflower seeds showed highest TAC on a DB (25566 μg Trolox g^{-1}, Fig. 2B) and specific TAC (1731 μg Trolox mg^{-1} phenolics, Fig. 2C). Those values were higher than others published in the literature, including red sweetpotato, purple corn and blueberry (Cevallos-Casals & Cisneros-Zevallos, 2003). Seven day sunflower sprouts showed significantly higher TAC on a DB (40202 μg Trolox g^{-1}) than the other seeds (1456–25991 μg Trolox g^{-1}) and than that of a blueberry reference (35232 μg Trolox g^{-1}), considered to have one of the highest antioxidant activities among fruits and vegetables (Cevallos-Casals & Cisneros-Zevallos, 2003). Due to their high phenolic specific TAC values, sunflower seeds seem to possess phenolic compounds with molecular structures bearing a high number of DPPH reactive hydroxyl (OH) groups. The major phenolic compounds identified in sunflower seeds have been chlorogenic acid (55%), 1,4-di-*O*-caffeoylquinic acid or 1,5-di-*O*-caffeoylquinic acid (chlorogenic acid derivative) (30%), caffeoyl-pentahydroxycinnamoyl-quinic acid (chlorogenic acid derivative) (10%) and caffeic acid (4%) (Pedrosa et al., 2000). Chlorogenic acid has been determined to have six reactive OH groups, a reactivity level higher than most phenolic compounds (Rice-Evans & Miller, 1996; Rice-Evans, Miller, & Paganga, 1996). It is possible that the functional groups of the two chlorogenic acid derivatives further enhance the molecular reactivity towards free radicals. According to Rice-Evans et al. (1996), at least two or three neighboring phenolic OH groups and a carbonyl group in the form of an aromatic ester, o-lactone, or a chalcone, flavanone or flavone are essential for achieving a high level of antioxidant activity.

Other seeds showing high phenolic content and TAC included radish, broccoli, mustard and fava (Figs. 1 and 2). Radish and broccoli had the highest phenolic concentration on a WB (Fig. 1C) and on a DB (Fig. 1B) among dormant seeds. Broccoli and mustard had the highest phenolic concentration on a DB among imbibed seeds and 7d sprouts (Fig. 1B).

The amount of phenolics and TAC on a PSB at all germination stages was highest for fava bean, due to its large size (~1.5 g/seed) (Figs. 1A and 2A; Table 1). Even though sunflower was ranked fourth in total weight per seed, it was ranked second, following

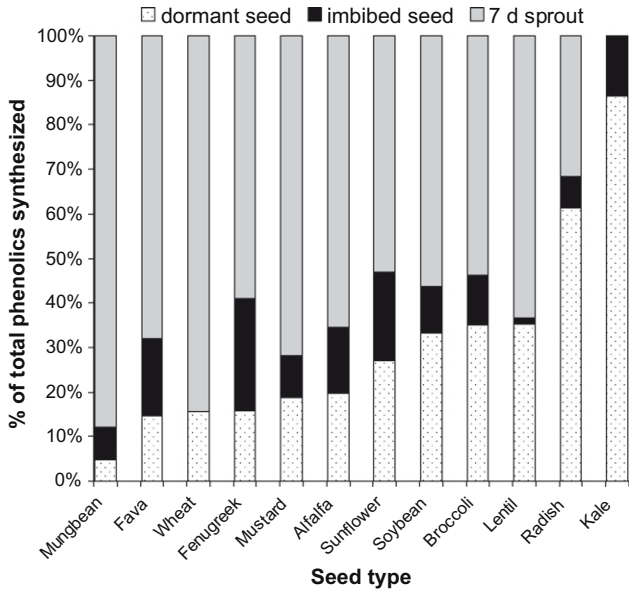

Fig. 3. Percentage of phenolics synthesised at the different germination stages: dormant stage, imbibition stage and 7d sprout stage.

Table 1
Changes in seed weight and moisture throughout germination at 18 °C.

Seed	Weight[a] (mg)			% Moisture		
	Dormant seed	Imbibed seed	7d Sprout	Dormant seed	Imbibed seed	7d sprout
Fava	1503 ± 21	2860 ± 205	3452 ± 946	7.4	62.7	71.5
Sunflower	64 ± 5	120 ± 1	307 ± 1	4.6	55.4	82.4
Soybean	222 ± 7	499 ± 31	622 ± 101	4.8	66.7	74.9
Mungbean	79 ± 3	176 ± 4	447 ± 3	6.4	58.5	85.2
Radish	13 ± 0.6	31 ± 1	83 ± 7	3.8	62.8	85.4
Fenugreek	20 ± 1	66 ± 2	153 ± 2	5.8	76.5	90.1
Broccoli	4.4 ± 0.3	8.8 ± 1	45 ± 1	3.7	60.3	92.8
Lentil	29 ± 1	81 ± 12	130 ± 5	7.3	70.7	71.9
Wheat	25 ± 2	40 ± 3	99 ± 9	15.4	57.0	86.3
Kale	4.8 ± 0.0	9.6 ± 0.4	11 ± 1	3.7	53.9	72.1
Mustard	2.2 ± 0.0	6.8 ± 1	19 ± 2	4.9	76.7	94.6
Alfalfa	2.1 ± 0.0	8.3 ± 0.3	19 ± 1	10.0	77.5	93.9
Onion	4.1 ± 0.1	9.4 ± 1	15 ± 0.1	5.7	57.1	72.6

[a] Values for weight show the average of 3–6 replicates ± standard deviation.

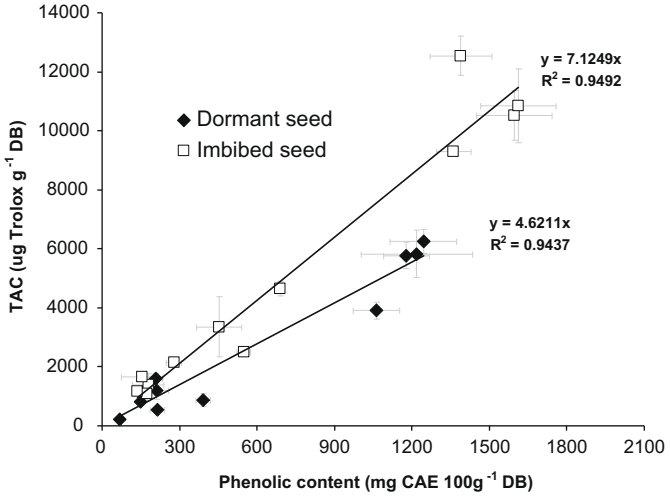

Fig. 4. Increase in antiradical efficiency during seed imbibition. Lines show linear regression fittings of the data. Data shows all seeds except sunflower. Both curves significantly different at $\alpha = 0.01$ using ANCOVA (testing for slopes). Values show the average of 3–6 replicates ± standard deviation.

fava, in amount of phenolics and TAC on a PSB at different stages tested (Figs. 1A and 2A; Table 1).

In the present study, phenolic contents of 7d sprouts on a WB, DB, and PSB ranged from 122 (alfalfa) to 555 (sunflower) mg CAE 100 g^{-1}, from 490 (lentil) to 5676 (mustard) mg CAE 100 g^{-1}, and from 0.02 (alfalfa) to 6.4 (fava bean) mg CAE $seed^{-1}$, respectively. On the other hand, blueberries have been reported to contain phenolic concentrations ranging from 292 to 672 mg CAE 100 g^{-1} on a WB and from 1956 to 4202 mg CAE 100 g^{-1} on a DB (Cevallos-Casals & Cisneros-Zevallos, 2003). The high level of phenolic antioxidants in most of the sprouts tested suggests that consumption of fresh sprouts could potentially provide similar antioxidant benefits to those of fresh blueberries. In addition, potential applications of germination include production of nutraceutically enhanced seeds for animal feed, for value-added nutraceutical extracts, or for value-added seed by-products such as soybean products (i.e. soy milk, soy sauce, tofu, okara) or malting products (i.e. beer, whisky). Innumerable commercial applications could be applied for mature sprouts as well.

4. Conclusions

From the above discussion it may be concluded that at initial germination stages phenolics may serve as radical scanvengers or antioxidants, while later they could become part of the structural framework of the growing plant and lose some of their antioxidant efficiency. Accumulated phenolics and TAC on DB showed the general trend distribution of 7d sprouts > dormant seeds > imbibed seeds. The phenols synthesised during seed germination could help obtain enhanced levels of phenols and antioxidant activity resulting in their improved nutraceutical properties. Seed germination could also serve as models for applying similar nutraceutical-enhancement strategies to other crops.

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