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Effects of germination on total phenolic compounds and radical scavenging activity in hull-less spring cereals and triticale

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Abstract. The aim of the current research was to evaluate changes in the content of total phenolic compounds and radical scavenging activity at different germination stages of triticale, hull-less barley, hull-less oats, wheat, and rye. Grain germination was performed for 12, 24, 36 and 48 h at controlled conditions. Ultrasound assisted extraction was used for isolation of total phenolic compounds. For all extracts the total phenolic compounds content, DPPH radical and $ABTS^+$ radical scavenging activity were determined spectrophotometrically. The results of the experiments demonstrated that the highest content of total phenolic compounds and the highest antiradical activity was determined in hull-less barley samples. In all studied grains the content of phenolic compounds increased significantly during soaking and germination process. DPPH radical scavenging activity during germination increased. $ABTS^+$ radical scavenging activity also increased after soaking process and dynamics were cereal type dependent. Pearson's coefficients between the phenolic compound levels and antioxidant activity taking into account all obtained results were high. Very strong positive correlations between the content of phenolic compounds and antioxidant activity were determined for triticale during germination. Also antioxidant activity determined by both tests correlated with the highest results for barley and oats. The highest content of total phenolic compounds determines the optimum duration of germination to be 24 hours, except rye samples where the highest value was reached only after 48 hours of germination. Shorter time was required to reach the highest values of DPPH radical activity – for wheat rye, and triticale, namely 12 hours.

Key words: cereals, germination, total phenolic compounds, radical scavenging activity.

INTRODUCTION

Grains and related processed products are consumed globally as important energy sources. Grain-based foods provide the majority of the carbohydrates, some proteins, lipids, dietary fiber, and other micronutrients in many diets (Luthria et al., 2015). Human diet includes the most important grains – wheat, rice, and corn, which are supplemented by other minor grains – oats, barley, rye, triticale, sorghum, millet, and buckwheat. It is well documented that germinated cereals have a greater nutritive and physiological value than un-germinated cereal and pseudocereal grains and their products (Prodanov et al., 1997; Price, 1988; Rozan et al., 1999; Rozan et al., 2000; Donkor et al., 2012). These

modified (germinated) grains, with increased levels of bioactive compounds, may have capacity in combating burning health issues among the obese and diabetics, as well as the potential to reduce the risk of colon cancer (Donkor et al., 2012).

The bioactive phytochemicals in wheat (*Triticum aestivum* L.) can be broadly subdivided into the following categories: phenolic acids, carotenoids, tocopherols, alkylresorcinols, and other miscellaneous compounds (sterols, steryl ferulates, benzoxazinoids and lignans) (Luthria et al., 2015). Wheat is commonly processed prior to consumption. Grain producers, processors, consumers, health, and nutritional professionals are interested in investigating the effect of processing on bioactive phytochemicals present in wheat (Wang et al., 2014). Rye (*Secale cereale* L.) has traditionally had an important role in the daily diet especially in Northern and Eastern Europe, and in addition to dietary fibre, rye grain is a good source of various phytochemicals (Pihlava et al., 2015) and be exploited more efficiently in new types of cereal products due to its positive health effects. Nowadays, its use is limited mainly due to the problems arising from its flavour; not all European consumers are familiar with the somewhat foreign, rye-like flavour, perceived as bitter and intense (Heiniö et al., 2003). Among the phytochemicals in rye, phenolic acids, alkylresorcinols and lignans, in particular, have gained a special focus (Bondia-Pons et al., 2009). Triticale is a hybrid of wheat (*Triticum*) and rye (*Secale*). Triticale (X *Triticosecale* Whittmack) is a potential alternative to wheat in processed flour products such as bread, flat bread, cakes or pasta. Triticale is rich in phenolics and dietary fibres consisting of both soluble and insoluble fibres (Agil & Hosseinian, 2014). Barley (*Hordeum vulgare* L.) has been intensively investigated in respect to its food, feed and industrial applications. Of the various barley cultivars, hull-less barley has recently been receiving considerable attention concerning the development of functional food, as it is an excellent source of both soluble and insoluble fibre. Hull-less (or 'naked') barley *(Hordeum vulgare* L. var. *nudum* Hook. f.) is a form of domesticated barley, in which, unlike hulled barleys but similarly to wheat, the lemma and palea (hull) are non-adherent to the caryopsis (Blandino et al., 2015). The advantage of hull-less barley compared to hulled barley in food uses is that pearling is not needed, so that the outer part of the endosperm, the aleurone, which contains proteins with essential amino acids and vitamins, is retained, as well as other bioactive compounds (Andersson et al., 2004). The nutritional improvement of hull-less oat relates to relatively high, energy rich oil content along with high protein content with a good balance of the amino-acids lysine, methionine and cysteine (Stroh et al., 2006). The most important bioactive compounds of oats (*Avena sativa* L.) are phenolic compounds. Some oat phenolics have great potential as nutraceuticals while some others are powerful antioxidants (Kilci & Gocmen, 2014). Oats contain antioxidants, and oat lipids are stable in mature, undamaged grains and in sufficiently heat-treated oat products (Molteberg et al., 1995).

Phenolic compounds are considered as a major group of compounds that contribute to the antioxidant activity of cereal. Moreover, upon germination the concentrations of these antioxidants increase. These molecules are secondary metabolites of plants possessing some positive physiological effects (Peng et al., 2015). Dietary antioxidants may play a significant role in human health preventing radical damage to biomolecules such as DNA, RNA, proteins, and cellular organelles. Therefore, there is increasing interest in identifying and assessing commonly consumed foods. The antioxidant activity of polyphenols has been mainly related to their redox properties, which can play an

important role in neutralizing free radical and quenching oxygen or decomposing peroxides (Kahkonen et al., 1999). Phenolic compounds are mainly concentrated in the bran fraction and covalently bound to indigestible polysaccharides (Wang et al., 2014). Due to hindrance by cereal matrices, most of the bound phenolic compounds are not accessible to attack by enzymes in the human gastrointestinal tract, leading to a low bioavailability. However, bioavailability of the bound phenolic compounds could be enhanced by increasing their accessibility primarily through particle size reduction, structural breakdown of cereal matrices, and their liberation from cereal matrices using suitable processing technologies (Wang et al., 2014).

Germination, a complex process causing physical, chemical and structural changes in grains, has been identified as an inexpensive and effective technology for improving cereal quality (Wu et al., 2013). The germination process is characterized by the growth of the embryo of the grain, manifested by the rootlets growth and increase in length of the shoot (acrospire), with the concomitant modification of the contents of the endosperm (Guido & Moreira, 2013). Factors influencing the germination include intrinsic parameters, such as cultivar or variety, and storage conditions, as well as external factors, such as temperature, humidity, presence of oxygen or air, light exposure and pH for germination (Cho & Lim, 2016).

During the process of germination significant changes in the biochemical, nutritional and sensory characteristics of cereals occur due to degradation of reserve materials as used for respiration and synthesis of new cell constituents for developing embryo in the seed. As compared to un-germinated seed, germinated seeds contain high protein, low unsaturated fatty acids, low carbohydrate, mineral content and vitamins (Narsih et al., 2012; Sharma et al., 2016). The phenols synthesised during seed germination could help in order obtain enhanced levels of phenols and antioxidant activity resulting in their improved nutraceutical properties (Cevallos-Casals & Cisneros-Zevallos, 2010). Intense biochemical processes occur during the grain activation (in the first stage of germination), as a result the content of vitamins B2, E and niacin, total sugar, dietary fibre and glucosamine increase; vitamin C is synthesized, and the content of essential amino acids is increased during the process of protein hydrolysis (Rakcejeva, 2007).

At the initial germination stages phenolics may serve as radical scavengers or antioxidants, while later they could become part of the structural framework of the growing plant and lose some of their antioxidant efficiency (Cevallos-Casals & Cisneros-Zevallos, 2010). Several studies suggested that germination significantly improved the functional (Singkhornart et al., 2014) and sensory (Ohtsubo et al., 2005; Singkhornart et al., 2014) properties of cereals and they can be used as a new approach to further developmentof a potential cereal products for human consumption.

The aim of the current research was to evaluate changes in the content of total phenolic compounds and radical scavenging activity at different germination stages of triticale, hull-less barley, hull-less oats, wheat, and rye.

MATERIALS AND METHODS

Plant material

The grains of conventionally grown hull-less barley (cv ‘Irbe’), hull-less oat (cv ‘Lizete’), rye (cv ‘Kaupo’), and wheat (cv ‘Elvis’) at State Priekuli Plant Breeding

Institute in Latvia and triticale (cv 'Tulus') cultivated at Norwegian Institute for Agricultural and Environmental Research (Norway) were tested. The experiments were carried out at the scientific laboratories of the Faculty of Food Technology at Latvia University of Agriculture.

Germination and sample preparation

The grains of all cultivars were cleaned, washed and soaked in water at the ratio of 1 : 2 (grains to water) for 24 ± 1 h at 22 ± 2 °C. After soaking, water was drained and grains were placed for germination in the climatic chamber ICH110 (Memmert, Germany) at controlled temperature (35 ± 1 °C) with relative humidity (RH) 95 ± 2% in the dark. Duration of the germination was 12, 24, 36, and 48 hours. Thereafter, the germinated grains were ground in a laboratory mill KN 195 Knifetec™ (Foss, Denmark) and analysed. Moisture content of germinated cereals was determined according to the AACC method 44-15A, which includes moisture removal at 135 °C for 90 min (AACC, 2000).

As a control grain sample un-soaked and un-germinated grains were tested.

Chemical analysis

Extraction of phenolic compounds from grains

The homogenized grain samples (2.0 g) were extracted with ethanol/acetone/water (7/7/6 v/v/v) solution in an ultrasonic bath YJ5120-1 (Oubo Dental, USA) at 35 kHz for 10 minutes at 20 ± 1 °C temperature. The extracts were then centrifuged in a centrifuge CM-6MT (Elmi Ltd., Latvia) at 3,500 min^{-1} for 5 min. Residues were re-extracted using the same procedure. The extraction process was done in triplicate.

Determination of total phenolic compounds

The total phenolic content (TPC) of the grain extracts was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999). To 0.5 ml of extract 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) was added and, after 3 minutes 2 ml of sodium carbonate water solution (Na2CO3) (75 g l^{-1}) was added. The sample was mixed. After 30 minutes of incubation at room temperature, the absorbance was measured at 765 nm. Total phenols were expressed as gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of the samples. The absorbance was measured at 765 nm and total phenols were expressed as the gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of grain material.

Determination of antioxidant activity

Antioxidant activity of the grainextracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl (DPPH) radical as outlined by Yu et al. (2003). The absorbance was measured at 517 nm. The radical scavenging activity of extract was also measured by 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic) acid ($ABTS^{+}$) radical cation assay (Re et al., 1999). For the assessment of extracts, the $ABTS^{\bullet+}$ solution was diluted with a phosphatebuffersolution to obtain the absorbance of 0.800 ± 0.030 at 734 nm. The radical scavenging activity was expressed as Trolox equivalents (TE) 100 g^{-1} DW of plant material.

Statistical analysis

Experimental results were means of three replications and were analyzed by Microsoft Excel 2010 and SPSS 17.00. Analysis of variance (ANOVA) and Tukey's and Pearson`s tests were used to determine differences among samples. A linear correlation analysis was performed in order to determine relationship between TPC, antioxidant activity such as $DPPH^{\bullet}$, $ABTS^{\bullet+}$ scavenging activity. Differences were considered as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Total phenolic content

Soaking and germination process influenced significantly the total phenolic content (TPC) of all tested grain types (Fig. 1). In soaked grains TPC generally increased, with exception of oat grain where significant decrease was observed. The TPC did not change significantly after 12 h of germination in wheat and barley samples, decreased in rye and triticale and increased in oat samples. Prolonged germination time mainly increase the content of TPC. Significant decrease occurred in TPC content after 48 hours in barley and wheat samples. Increase of TPC most probably occurs due to distribution of phenolic acids during germination process by starch enzymatic hydrolyses (Maillard et al., 1996; Tian et al., 2004). Chen et al. (2016) also studied changes in the total phenolic content of canary seeds and reported a general trend – germinated seeds are reach in total phenolic then raw and soaked seeds (Chen et al., 2016). Another study revealed that in different seeds accumulated phenolics and antioxidant activity showed the general trend distribution of 7 day sprouts > raw seeds > steeped seeds (Cevallos-Casals & Cisneros-Zevallos, 2010).

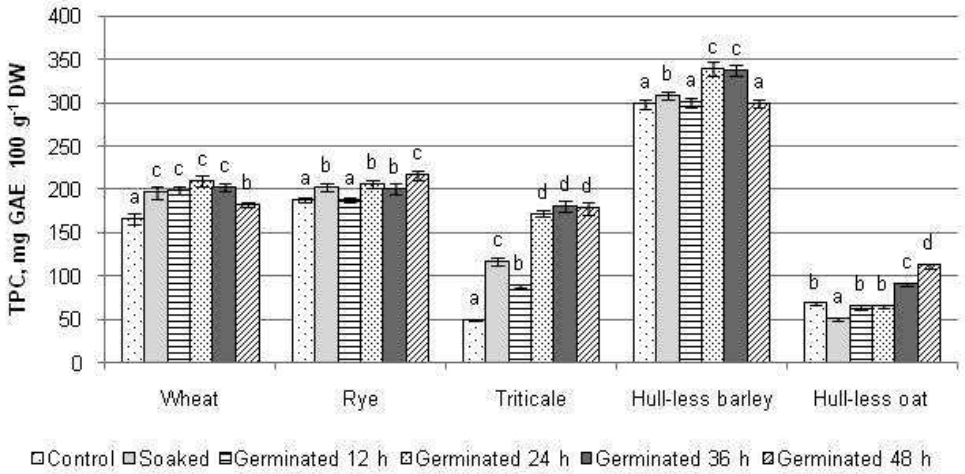

Figure 1. Dynamics of TPC during germination process of wheat, rye, triticale, hull-less barley and hull-less oat. Note: the values marked with different letters for each cereal type represent significant differences between values ($P < 0.05$).

Our study shows that the content of TPC increased significantly for all types of cultivars after 24 hours, except hull-less oats. It seems that oats need germinating at least for 36 hours or even more to enhance TPC content significantly. Tian et al. (2010) reported more than a 4-fold increase in phenolic compound contents in the oat after germination for 120 h. Probably, 48 h germination period for hull-less oats was too short in order to release phenolic compounds, and therefore optimization of germination process is necessary to obtain maximum content of phytochemicals. All germinated grains contained increased amounts of total phenolic content with hull-less barley having significantly ($P < 0.05$) higher content compared with the non-germinated grains (Fig. 1). The smallest increase of TPC during germination among studied grains was found in wheat and rye, while the biggest increase, approximately 2.5 times, was observed in triticale. Phenolic compounds present in cereal grains would contribute to functional and nutritional properties of the grain (Tian et al., 2010), thus increasing their nutritional value. Other studies suggested that apart from increasing total phenolic content, significant improvement in Vitamin E content has been observed for various germinated cereal grains (Kim et al., 2012; Žilić et al., 2013), primarily due to the generation of a variety of bioactive components including tocopherols and tocotrienols (Moongngarm & Saetung, 2010) and/or liberation of bound Vitamin E homologues from cellular components in grains during germination processes (Ng et al., 2013).

The degree of the changes seen in chemical composition depends on various germination conditions, such as temperature, humidity, soaking and the length of germination (Rakcejeva, 2007). According to Donkor et al. (2012) this means direct comparison is difficult, and optimum conditions will need to be defined for individual cereals. At the same time with synthesis of novel compounds, the concentrations of some nutrient inhibitors may decrease.

Antioxidant activity

During soaking process the DPPH scavenging activity in grains increased significantly. During the following germination process dynamics of DPPH differed among cereal types (Fig. 2). Radical scavenging activity of the phenolic extracts for non-germinated grains were between 435 ± 16 mM TE 100 g^{-1} DW (triticale) and 680 ± 26 mM TE 100 g^{-1} DW (hull-less barley). For 24 hours germinated grains DPPH scavenging activity ranged between 581 ± 14 mM TE 100 g^{-1} DW (wheat) and 1232 ± 17 mM TE 100 g^{-1} DW (hull-less barley). Even though all the grains showed substantial DPPH radical scavenging activity, hull-less barley in the germinated form appeared to have exhibited the highest activity.

During germination hydrolytic enzymes modify endosperm of components with antiradical activity (Sharma & Gujral, 2010). Enzymatic release of bound phenolics increases the TPC values during malting of barley as well as their antioxidant properties (Dvorakova et al., 2008). Cevallos-Casals & Cisneros-Zevallos (2010) repoted that significant increase of DPPH activity was determined after soaking process, whereas content of phenolic compounds in steeped seeds was lower than in raw seeds. The current research demonstrated an increase in wheat DPPH activity, with the highest activity after 12 h of germination. Hung et al. (2011) also reported that during germination wheat exhibited an increase in their antioxidant activities. The highest DPPH radical scavenging activity was detected in hull-less barley, which was in line with the study of Žilić et al. (2011) who detected higher DPPH radical scavenging ability in hull-less

barley, followed by rye and hull-less oat and durum and bread wheat, indicating that small grain species have different major antioxidants with different properties. According to Alvarez-Jubete et al. (2010) germination increase antioxidant capacity, however this may depend on cereal type, crop variety as well as of germination conditions (Gallegos-Infante et al., 2010).

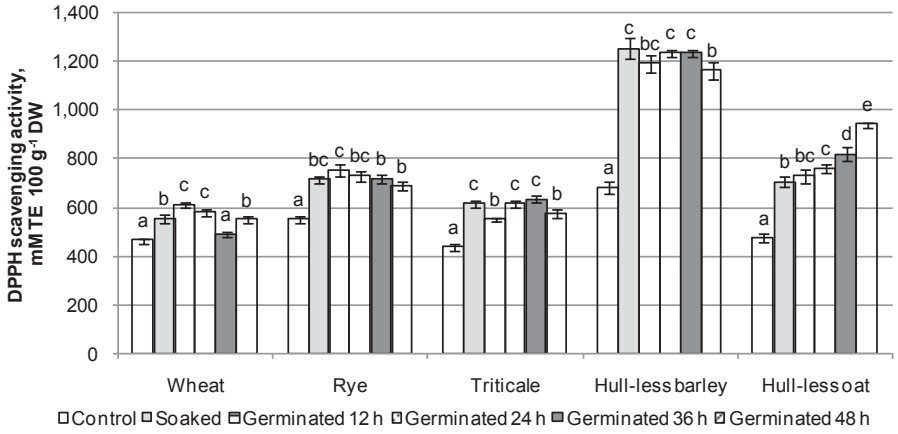

Figure 2. Dynamics of DPPH radical scavenging activity during germination process. Note: the values marked with different letters for each cereal type represent significant differences between values ($P < 0.05$).

ABTS radical scavenging activity after soaking process also increased significantly (Fig. 3). After 12 h of germination significant decreases were observed, except rye where decrease was measured after 24 h. In general hull-less barley grains had the highest ABTS radical scavenging activity.

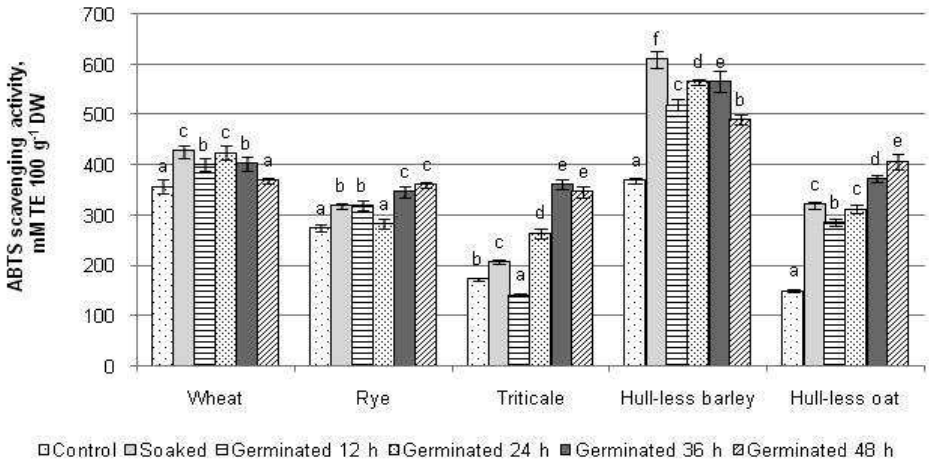

Figure 3. Dynamics of ABTS cation scavenging activity during germination process. Note: the values marked with different letters for each cereal type represent significant differences between values ($P < 0.05$).

Relationships between phenolic compounds and antioxidant capacity

Wheat, rye and hull-less barley grains containing higher levels of phenolic compounds also displayed higher scavenging activity. Similar results were reported previously (Donkor et al., 2012). However, hull-less oats had relatively low TPC among studied cereals (Fig. 1), but oat grain DPPH radical scavenging activity was comparable with activities of rye and triticale (Fig. 3). Thus radical scavenging activity in hull-less oats may be provided by phenols in combination with other compounds. Masisi et al. (2016) indicated that wide range of phytochemicals has been recognized to support overall health through their antioxidant potential. Whole grain cereals are good sources of phenolic compounds which include derivatives of benzoic and cinnamic acids, anthocyanidins, quinines, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds, grains contain tocotrienols and tocopherols, and oryzanols which have antioxidant properties.

Pearson's coefficients between the phenolic compounds levels and antioxidant activity separately in each cereal type and also total correlation taking into account all obtained results are presented in Table 1.

Table 1. Correlation between TPC, DPPH radical scavenging activity, ABTS cation scavenging activity in grains

Cereal	Pearson's correlation coefficient		
	TPC/DPPH	TPC/ABTS	DPPH/ABTS
Wheat	0.59*	0.89**	0.55
Rye	0.31	0.55	0.45
Triticale	0.82*	0.89**	0.56*
Hull-less barley	0.48	0.56	0.94**
Hull-less oats	0.65*	0.56	0.98**
All cereals	0.63**	0.79**	0.77**

TPC/DPPH – correlation between total phenolic content and DPPH radical scavenging activity;
TPC/ABTS – correlation between total phenolic content and ABTS radical scavenging activity;
DPPH/ABTS – correlation between DPPH radical scavenging activity and ABTS radical scavenging activity;
* correlation is significant at $p < 0.05$;
** correlation is significant at $p < 0.01$.

On average for all grain cultivars the Pearson's coefficients between the phenolic compound levels and antioxidant activity were high (TPC with DPPH and ABTS respectively $r = 0.63$ and $r = 0.79$). We found very strong positive correlations ($r = 0.82$ and $r = 0.89$) between both the content of phenolic compounds and antioxidant activity in triticale. Similar results were obtained by Bleidere et al. (2013) showing significant ($P < 0.05$) positive correlation between radical scavenging activity and total phenolic content ($r = 0.519$) in hulled barley. And also Zhao et al. (2008) reported that total phenolic content showed strong correlation with DPPH radical scavenging activity in spring barley. Opposite results were obtained by Dordevic et al. (2010) who did not find correlation between TPC and DPPH scavenging activity in the grains. Very strong correlation between TPC and ABTS scavenging activity ($r = 0.971$) was reported for commercial canola meal (Hassas-Roudsari et al., 2009) and durum ($r = 0.950$) (Žilić et al., 2012). Whereas Italian researchers analysing whole grain durum wheat (*T. durum* Desf.) determined strong correlation ($r = 0.663$) (Laus et al., 2012). Also antioxidant activity determined by both tests in our study correlated with the highest results for hull-

less barley ($r = 0.94$) and hull-less oats ($r = 0.98$) which allows to predict that an increase in one indicator results in increase in other indicators.

CONCLUSIONS

The germinated grains contained significantly more phenols than non- germinated grains. The highest amounts of phenols were measured in hull-less barley grains and it was significantly higher ($P < 0.05$) than in the grains of wheat, rye, triticale and hull-less oats. However, in triticale and hull-less oats increase in phenol compounds was the highest.In order to obtain the highest TPC and antioxidant activity the optimum germination time was 24 h. The closest correlation between TPC, DPPH radical scavenging activity, ABTS cation scavenging activity was recognised in hull-less barley grains.

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