

Synthesis of Antioxidants in Wheat Sprouts

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Aqueous and ethanolic extracts from wheat (*Triticum aestivum*) sprout powder were analyzed to determine its reduction and antioxidant activities. Mean and standard deviation of five independent samples were reported. The results showed that the micromoles of potassium ferricyanide reduced by aqueous and ethanolic extracts corresponding to 1 g of sprout powder (80.6 ± 11.2 and 9.7 ± 1.8 , respectively) were higher than that reduced by 1 mg of other reducing compounds: ascorbic acid, rutin, quercetin, and reduced glutathione (4.8 ± 0.7 , 3.8 ± 1.2 , 4.8 ± 1.7 , and 1.6 ± 0.4 , respectively). In addition, the oxygen superoxide scavenging activity performed by sprout extracts (from 1 g of powder) is comparable to that shown by 10 mg of antioxidant pure compounds (rutin and quercetin). Biochemical analysis of the sprout extracts shows that the antioxidant activity is mainly due to reducing glycoside and polyphenolic compounds.

KEYWORDS: Oxidative stress; wheat sprout extracts; antioxidants; radical scavenger activity

INTRODUCTION

Although at moderate concentrations, nitric oxide (NO), superoxide anion and related reactive oxygen species (ROS) play an important role as regulatory mediators in signaling processes, at high concentrations these reactive molecules are hazardous for living organisms and damage all major cellular constituents (1). An excessive and/or sustained increase in ROS production has been implicated in the pathogenesis of cancer, diabetes mellitus, atherosclerosis, and neurodegenerative and other diseases. Moreover, the process of aging may result, at least in part, from radical-mediated oxidative damage (2).

Accordingly, in the past two decades an increasing interest has been focused on the study of natural products with antioxidant activity. Beneficial effects of fruits and vegetables, such as protection against aging and several human diseases, have been described by many authors. Eicholzer et al. (3) reported that diets rich in fruit and vegetables may be powerful in the prevention of cardiovascular disease. Positive effects of natural antioxidant products on cataract and ocular diseases have been demonstrated by Anderson et al. (4), Taylor et al. (5), Niwa et al. (6), and Orhan et al. (7). Antioxidants may contribute to prevent cancer (8, 9) and delay aging (10).

As far as the potential functional peculiarities acquired by vegetal seeds during the germination process are concerned, it has been reported that aqueous extracts from wheat sprouts inhibit the mutagenic effect induced by benzo[*a*]pyrene in strain TA98 of *Salmonella typhimurium* (11, 12). Moreover, through

induction by benzo[*a*]pyrene of sperm abnormalities in mice, these abnormalities were found to diminish after oral administration of wheat sprout extract (13). We have previously shown that wheat sprouts contain antioxidant compounds active in the protection of DNA against the oxidative stress induced by Fenton reaction ($\text{Fe}^{2+}/\text{H}_2\text{O}_2$) (14). Likewise, Brussels sprouts contain bioactive substance(s) with a potential for reducing the physiological, as well as oxidative stress-induced, DNA damage in rats and humans (15, 16).

In this paper we report the results of the analysis carried out on wheat sprout extracts to assay the activity of some redox enzymes and to detect low molecular weight antioxidant compounds. Reduction and antioxidant activities of wheat sprout aqueous and ethanolic extracts have been measured.

MATERIALS AND METHODS

All reagents were of pure analytical grade. Hypoxanthine, xanthine oxidase from bovine erythrocytes, phosphomolybdic acid, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), potassium ferricyanide, and nitrotriazolium blue chloride (NBT) were obtained from Sigma Chemical Co., St. Louis, MO.

The following antioxidant standards were utilized in the course of the experiments: 1, reduced ascorbic acid; 2, quercetin; 3, rutin; 4, reduced glutathione; 5, coenzyme Q₁₀. These compounds were also obtained from Sigma Chemical Co.

Wheat (*Triticum aestivum*) from biological agriculture was utilized for all experimental procedures.

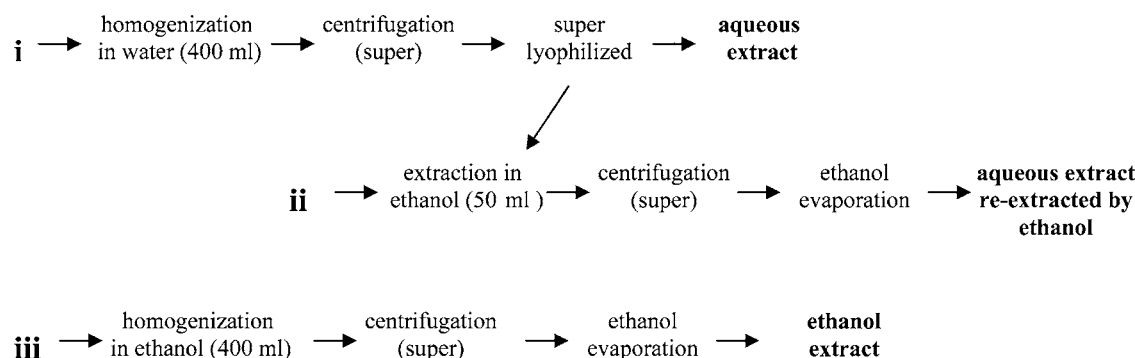
Wheat Sprout Powder. Wheat seeds were soaked overnight in water and subsequently sprouted for 3–5 days on sterilized soft agar (0.8–1%, in water) at 15–25 °C until the sprouts reached a length of 3–4 cm. The sprouted wheat was dehydrated by means of a continuous flow of dehumidified air at 15–20 °C. After dehydration, the sprouts were mechanically detached from the seeds by a cutter equipped with special blades suitable to achieve the result of detaching the sprout

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Scheme 1



from the seed and of partially breaking it without shattering the seed. The sprouts were separated from the seeds and the fragments of dehydrated agar (that have the appearance of a transparent film) by sieving. The isolated sprouts were then finely ground with a high-speed cutter provided with very sharp blades operating at 10 homogenization steps of 15 s each and alternating each step with a 15 s pause to prevent the material from heating. The so-obtained sprout powder was preserved in hermetically sealed vacuum vessels. The preparation of the sprout powder was carried out by taking into account the microbiological safety evaluations and recommendations on sprouted seeds (17).

Wheat Sprout Powder Extracts. Extracts from 20 g of sprout powder were prepared by three procedures (see Scheme 1).

(i) *Total Aqueous Extract.* Sprout powder was suspended and homogenized (by means of a Waring Blender) in 400 mL of water and centrifuged at 10000g for 30 min at 4 °C. The supernatant was lyophilized.

(ii) *Ethanol-Extracted Aqueous Extract.* The total aqueous extract was re-extracted with 50 mL of 100% ethanol, stored at 4 °C for 2 h, and centrifuged at 10000g for 30 min at 4 °C. The ethanol was then removed by evaporation, and the residue was resuspended in 15 mL of water and lyophilized.

(iii) *Ethanolic Extract.* Sprout powder was suspended and homogenized (by means of a Waring Blender) in 400 mL of 100% ethanol and centrifuged at 10000g for 30 min at 4 °C. After storage at 4 °C for 24 h, the extract was centrifuged at 10000g for 30 min at 4 °C; then the supernatant was evaporated, and the residue was resuspended in 15 mL of water and lyophilized.

All of the dried extracts (i, ii, and iii) after the final lyophilization were resuspended in water at concentrations varying from 5 to 100 mg/mL (w/v), according to requirements of the various experiments. The extract concentrations are specified in the figure captions.

Control extracts were obtained from sprouted seeds (20 g) ground after the sprout detachment and from unsprouted ground seeds (20 g). Both samples were treated in the same way as the sprout powder.

Catalase Activity. The presence of catalase in the wheat sprout extract was qualitatively observed by following the appearance of gaseous oxygen after the addition of H₂O₂.

Peroxidase Activity. The peroxidase activity was measured by the absorbance increase at 460 nm of *o*-dianisidine in the presence of hydrogen peroxide (18).

Cytochrome *c* Reductase Activity. The cytochrome *c* reduction by wheat sprout extracts was monitored by the absorbance increase at 550 nm.

Thin-Layer Chromatography (TLC). TLC was performed on silica gel plates using different solvent systems. After drying, the plates were sprayed with 10% phosphomolybdic acid solution in ethanol (w/v) and heated at 120 °C until spot formation was obtained (19). Phosphomolybdic acid in the presence of reducing substances is transformed into molybdenum blue, which is visible on the TLC sheet as a blue spot. In some experiments, the plates were sprayed with Folin–Ciocalteu reagent to detect phenols or orcinol–ferric chloride–sulfuric acid and *p*-anisaldehyde–sulfuric acid reagent to show the presence of mono- and oligosaccharides.

Analysis of Polyphenol Content. Measurement of the phenol and polyphenol contents was performed with phosphomolybdic–phospho-

tungstic acid reagent, according to the method of Singleton et al. (20). The values have been computed by utilizing as standard the dose–response obtained with gallic acid.

Analysis of Flavonoid Content. Measurement of the flavonoid content was performed with aluminum chloride reagent, according to the method of Chang et al. (21). The values have been computed by utilizing as standard the average of the dose–responses obtained with apigenin, quercetin, and rutin.

Analysis of –SH Groups. Measurement of the –SH group content was performed with DTNB reagent, according to the method of Ellman et al. (22). The values have been computed by utilizing as standard the dose–response obtained with reduced glutathione.

Reducing Power of Sprout Extracts. The total reducing power of sprout extracts was measured by utilizing potassium ferricyanide as reagent, following the method of Yen and Chen (23). The samples (containing different amounts of extract) were mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6, and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. An equal volume of 1% trichloroacetic acid was then added to the mixture, which was then centrifuged at 6000 rpm for 10 min. The upper layer of solution was mixed with distilled water and 0.1% FeCl₃ at a ratio of 1:1:2, and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. An absorbance increase of 1 unit corresponds to the reduction of 0.5 μmol of potassium ferricyanide.

$$\mu\text{mol of reduced potassium ferricyanide} = \frac{1}{2} \times \text{OD} (700 \text{ nm})$$

NBT (Superoxide Scavenging) Assay. Measurement of superoxide radical scavenging activity was carried out on the basis of the method described by Kirby and Schmidt (24). Twenty-five microliters of 15 mM Na₂EDTA in buffer (50 mM KH₂PO₄/KOH, pH 7.4), 62 μL of 0.6 mM NBT in buffer, 37 μL of 3 mM hypoxanthine in 50 mM KOH, from 12.5 to 50 μL of test samples in water, and 187 μL of buffer were mixed in 96-well microplates (Falcon). In the blank the extract was substituted by an equal volume of water. The reaction was started by adding 50 μL of xanthine oxidase solution in buffer (1 unit in 10 mL of buffer) to the mixture. The reaction mixture was incubated at 25 °C, and the absorbance at 570 nm was determined every 1 min in the first 5 min and then every 5 min up to 30 min using a plate reader (Labsystems Multiskan MS). The radical scavenging activity is measured as inhibition of NBT reduction. A decrease of 0.7 absorbance unit corresponds to 18.6 nmol of NBT not reduced by oxygen superoxide. Superoxide can reduce NBT to monoformazan via one-electron transfer, and this reaction can be monitored spectrophotometrically at 570 nm (25). By assuming that the stoichiometric ratio between oxygen free radicals and reduced NBT molecules is 1:1, an inhibition of 0.7 absorbance unit should correspond with the scavenging of 18.6 nmol of oxygen superoxide.

$$\text{nmol of scavenged oxygen superoxide} = 18.6 \times (\text{control sample OD} - \text{treated sample OD})/0.7$$

Fast Ion Bombardment Mass Spectrometry. Mass spectrometry analysis was performed on a VG/70 VSEQ instrument with an FIB

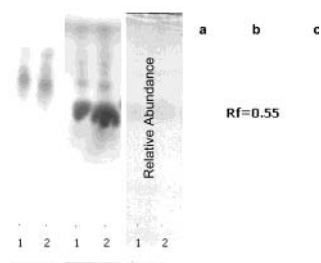


Figure 1. Ascendant TLC chromatography on silica gel plates (10×20 cm) of 2 (lane 1) and 4 μL (lane 2) of wheat sprout extract (50 mg/mL). Solvent system: propanol/water 70:30 (v/v). The plates were stained with Folin–Ciocalteu reagent (a), phosphomolybdic acid (b), and *p*-anisaldehyde/sulfuric acid reagent (c).

cesium ion gun, kinetic energy = 35 keV, emission current = 2 μA , resolution = 1000, positive ion mode; samples were prepared as an aqueous thioglycerol matrix.

RESULTS

Chemical analysis of the wheat sprout powder “in toto” shows values quite usual for vegetal tissues: humidity, 7.7%; ashes, 3.2%; proteins, 13.0%; lipids, 2.4%; soluble sugars, 5.4%; starch, 64.0%; fiber, 3.5% (total, 99.2%). However, the metal analysis shows an extraordinarily large amount of phosphorus (2.7%); interestingly, 1.6% is represented by organic phosphates. The zinc content is also noteworthy: 0.0128%.

Redox Enzymes in Wheat Sprouts. The presence of some redox enzyme has been evaluated in total aqueous extract (i) of wheat sprouts. The addition of hydrogen peroxide causes a dramatic formation of gaseous oxygen, thus demonstrating a clear presence of catalase. Moreover, it is noteworthy that 1 g of sprout powder contains 165 units of peroxidase and 1 unit of cytochrome *c* reductase. The activity of these enzymes is almost completely absent in the samples subjected to extraction with 100% of ethanol [extracts (ii) and (iii)] or in the aqueous extract (i) after heating at 100 °C for 2 h. The presence of superoxide dismutase has not been measured because we have observed that the extraction with absolute ethanol or the heating at 100 °C for 2 h reduces the radical scavenging activity of the extracts by <20%. These results demonstrated that the radical scavenging activity of wheat sprout extracts is mostly due to nonenzymatic molecules.

Low Molecular Weight Antioxidant Molecules. With regard to the presence of low molecular weight antioxidant molecules, TLC analysis of wheat sprout extracts shows, after staining with phosphomolybdic acid, a main spot with $R_f = 0.55$ (Figure 1b). We have previously reported that this compound is probably represented by an antioxidant glycoside and that it strongly increases during the germination phase of the wheat seeds (1–4 days of germination) (14). Accordingly, the presence of this compound is less in seeds after sprout detachment and almost completely absent in nonsprouted seeds (data not shown). Figure 1a shows a TLC loaded with the same samples and stained with Folin–Ciocalteu reagent, which reacts with phenols. The spots correspond to those reported in panel b and showing $R_f > 0.55$. The results reported in panel c confirm that the spot at $R_f = 0.55$ contains a glucide moiety. This fraction has been re-extracted from a preparative TLC and, following purification by G25 Sephadex column, analyzed by fast ion bombardment mass spectrometry (FIB⁺). The mass spectrum (Figure 2) shows a peak of 203 kDa to which three hexose residues are bound ($203 + 162 = 365 + 162 = 527 + 162 = 689$).

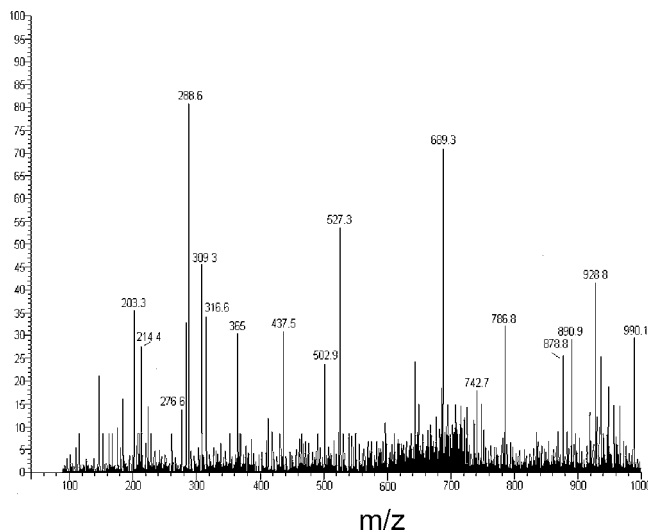


Figure 2. FIB⁺ mass spectrum of the fraction with $R_f = 0.55$ (Figure 1) re-eluted from preparative TLC.

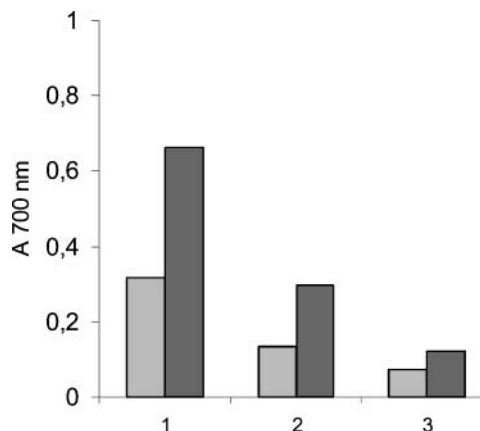


Figure 3. Total reducing power measured by potassium ferricyanide reagent: (1) wheat sprout extract (iii); (2) extract of wheat seeds after sprout detachment; (3) nonsprouted wheat seeds extract. Lighter shaded bars correspond to an extract concentration of 0.25 mg/mL, and heavily shaded bars correspond to an extract concentration of 0.5 mg/mL (final concentration).

Following the analytical results obtained by TLC, the polyphenol content in wheat sprout ethanolic extract (iii) has been measured according to the method of Singleton et al. (20). The results obtained in five independent samples show a value of 4.6 ± 1.1 mg/g of powder. Accordingly, within the family of reducing polyphenols, the flavonoids represent an interesting component of the antioxidant compounds synthesized during the germination process. In fact, measurement of the flavonoid content performed in the wheat sprout extract (iii) with aluminum chloride reagent (21) indicates a value of 3.3 ± 0.8 mg/g of sprout powder. The level of reduced –SH groups has been also measured in ethanolic sprout extracts (ii) and (iii) by utilizing reduced glutathione as standard. The content of –SH groups in the ethanolic extracts obtained from 1 g of powder is ~ 2 nmol.

Reducing Activity of Wheat Sprout Extracts. In Figure 3 the reducing powers of wheat sprout extract, sprouted seed extract, and nonsprouted seed extract are reported. The data demonstrate that wheat sprout extracts contain high levels of antioxidant molecules. Moreover, it is evident that the antioxidant activity of wheat seeds strongly increases during the germination process. We have also observed that the maximum

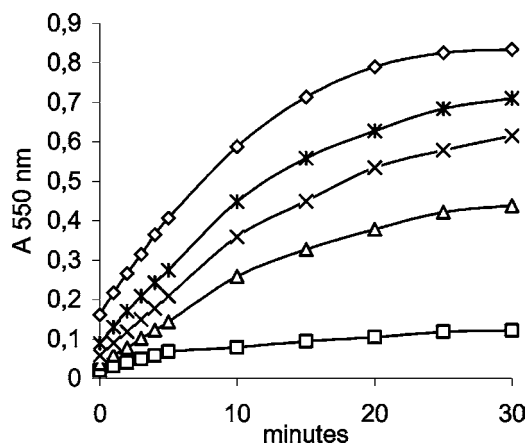


Figure 4. Kinetics of O_2^- scavenging activity of wheat sprout extracts (iii) (solubilized at a concentration of 100 mg/mL): control (\diamond); 6.25 μ L (*); 12.5 μ L (x); 25 μ L (Δ); 50 μ L of wheat extract (\square).

Table 1. Micromoles of Potassium Ferricyanide Reduced by Aqueous and Ethanolic Extracts from 1 g of Wheat Sprout Powder by 1 mg of Pure Reducing Compound^a

reducing compound	μ mol of reduced ferricyanide
aqueous extract (i)	80.6 \pm 11.2
aqueous extract re-extracted with ethanol (ii)	8.4 \pm 1.8
ethanolic extract (iii)	9.7 \pm 2.3
vitamin C	4.8 \pm 0.7
rutin	3.8 \pm 1.2
quercetin	4.8 \pm 1.7
coenzyme Q ₁₀	
reduced glutathione	1.6 \pm 0.4

^a Values are expressed as a mean of five independent measurements \pm SD.

antioxidant activity is present in the sprout extracts of wheat seeds germinated for 3–5 days (data not shown). **Table 1** shows the micromoles of potassium ferricyanide that may be reduced by the antioxidant compounds contained in 1 g of wheat sprout extract. The reducing activity exerted on the ferricyanide reduction by several known reducing compounds (see Materials and Methods) has been also measured. The results demonstrate that the total aqueous extract (i) exerts a reducing activity \sim 10 times higher than that exerted by the deproteinized alcoholic extracts (ii) and (iii), thus showing that probably some enzymatic proteic molecules may be involved in the ferricyanide reduction. In fact, several enzymes may catalyze the ferricyanide reduction such as the cytochrome *c* reductase (26, 27) or the lactate dehydrogenase (28). As far as the standard compounds are concerned, reduced ascorbic acid, rutin, quercetin, and reduced glutathione exert the reduction of ferricyanide, whereas coenzyme Q₁₀ seems to be almost completely inactive. In any case, it is noteworthy that all of the extracts obtained from 1 g of sprout powder show an activity higher than that exerted by 1 mg of pure reducing molecules.

Radical Scavenging Activity of Wheat Sprout Extracts.

The sprout extracts were then used for the study of their radical scavenging activity by superoxide scavenging NBT assay. The kinetics of NBT reduction by superoxide generated by hypoxanthine–xanthine oxidase, in the absence and in the presence of several amounts of wheat sprout extracts, are reported in **Figure 4**. The extracts show a strong superoxide scavenging activity. In **Table 2** the radical scavenging activity exerted by the antioxidant molecules contained in 1 g of sprout powder is reported. This activity is expressed as micromoles of NBT that

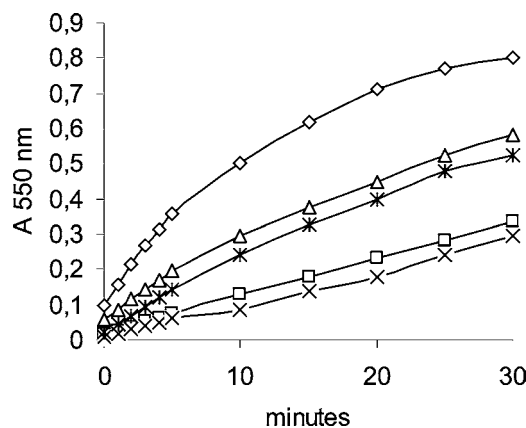


Figure 5. Kinetics of O_2^- scavenging activity of wheat sprout extracts (iii) (solubilized at a concentration of 100 mg/mL) before and after heating at 100 °C for 2 h: control (\diamond); 20 μ L of wheat extract (*) and 20 μ L of wheat extract after heating (Δ); 40 μ L of wheat extract (x) and 40 μ L of wheat extract after heating (\square).

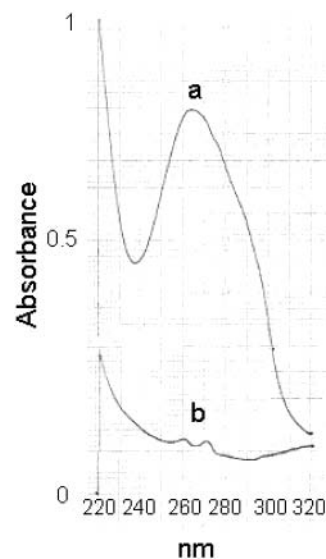


Figure 6. Absorption spectrum of wheat sprout extracts (iii) before (a) and after (b) adsorption on active charcoal.

Table 2. Radical Scavenging Activity Expressed as Inhibition of NBT Reduction^a

antioxidant compound	unreduced NBT (μ mol)
aqueous extract (i)	9.9 \pm 1.8
aqueous extract re-extracted with ethanol (ii)	7.6 \pm 0.9
ethanolic extract (iii)	7.8 \pm 1.4
rutin	17.2 \pm 3.0
quercetin	2.9 \pm 0.6

^a Activity of extracts from sprouts powder (1 g) and rutin (10 mg) and quercetin (10 mg) is reported. Values are expressed as mean of five independent measures \pm SD.

have not been reduced by superoxide. Aqueous and ethanolic wheat sprout extracts show very similar radical scavenging activities. These data demonstrate that this activity is mostly due to low molecular weight nonenzymatic molecules. Moreover, it is interesting that the antioxidant molecules contained in 1 g of sprout powder show a radical scavenging activity comparable to that exerted by 10 mg of pure reducing compounds (rutin and quercetin). An experiment has been carried out to demonstrate if this radical scavenging activity is

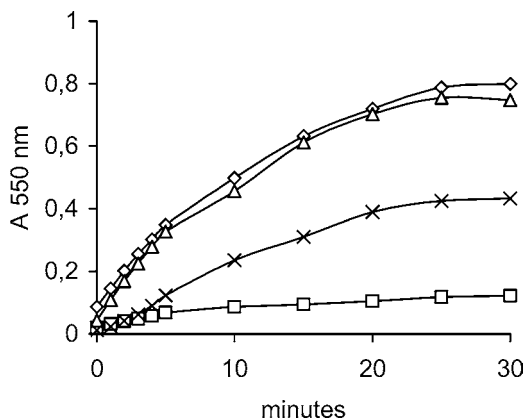


Figure 7. O_2^- scavenging activity of wheat sprout extract (iii) ($50 \mu\text{L}$ of a solution of 100 mg/mL) subjected or not to absorption on active charcoal and subsequent re-elution. Kinetics from the top to the bottom: control (\diamond); extract subjected to absorption on charcoal (\triangle); fraction after elution with hot ethanol (\times); untreated extract (\square).

due to thermostable or thermolabile molecules. The extracts were heated at 100°C for 2 h and subsequently their radical scavenging activities measured. The results indicated that the radical scavenging activity of the sprouts is quite thermostable (**Figure 5**).

Recently experiments have been carried out to separate the polyphenols and glycoside structures of sprout extracts by absorption on charcoal or X-Amberlite resin (Sigma). The results show that all of the substances containing aromatic structure are absorbed by charcoal. In fact, the absorption spectrum of the sprouts extracts from 220 to 320 nm is almost completely abolished following absorption on charcoal (**Figure 6**). Accordingly, the oxygen superoxide scavenging activity of the sprouts extracts is absent following treatment with charcoal (**Figure 7**), thus demonstrating that the sprout antioxidants have been absorbed by charcoal. Attempts to elute selectively the absorbed antioxidant molecules show that a significant fraction of the absorbed antioxidant compound may be re-eluted with hot (75°C) ethanol (**Figure 7**).

DISCUSSION

Our main research program has been focused for many years on the characterization of low molecular weight phosphopeptides. The peptide fraction isolated from the chromatin of several vegetal and animal tissues exerts a homeostatic regulation demonstrated as inhibition of DNA transcription *in vitro* and of fast-growing cancer cell proliferation and reactivation of some pathways slowed in senescence (29–32).

Recently we observed that wheat sprouts are a good source of the chromatin phosphopeptides (33). Sprouting wheat seeds show very strong activity of many kinases (for example, CKII kinase) from 1 to 4 days of germination, and the levels of organic phosphates are particularly high ($>1.6\%$). In this context we observed that another peculiarity of wheat sprouts is a strong antioxidant activity. In fact, the data described in this paper show that wheat sprouts contain a powerful cocktail of antioxidant molecules. We have previously reported that the level of the antioxidant activity of wheat sprouts is notably higher than that of wheat germ and young wheat plants (14). Accordingly, it has been shown that antioxidant activity is higher in wheat sprouts than in wheat seeds after sprout detachment and almost completely absent in nonsprouted wheat seeds. Wheat sprouts contain remarkable levels of some redox enzymes. In particular, catalase and peroxidase activities appear to be very strong. As far as low molecular weight antioxidant compounds are

concerned, a prominent fraction is shown by TLC stained with phosphomolybdic acid, which is probably represented by a reducing glycoside. The mass spectrometry analysis shows the presence of an ion at m/z 203 that should represent the aglycon structure of this reducing glycoside molecule ($MWH^+ = 689$). It may be noteworthy that several authors reported the presence of the ion at m/z 203 in the mass spectrum of flavonoid compounds (34). In particular, the possibility that this ion arises from the fragmentation of the derrone molecule induced by mass spectrometry analysis has been suggested (35). However, the antioxidant activity shown by this isolated fraction (MW 689) represents only 15–20% of the total activity exerted by the sprout extracts (data not shown). In fact, the presence of polyphenol fractions (**Figure 1**) and thiols has been also observed. The $-SH$ values obtained appear to be detectable but quantitatively not so relevant as to explain the strong antioxidant activity exerted by sprouts extracts.

On the other hand, a synergic effect by the different antioxidant compounds present in the wheat sprout may be hypothesized, keeping in mind that the antioxidant activity of a compound is dependent on its redox potential. Therefore, the presence of a series of compounds with different redox potentials could strengthen the protection ability against an oxidative insult. The antioxidant activity of wheat sprout extracts has been compared with that of some known antioxidant compounds. The comparison with rutin, quercetin, and reduced glutathione appears to be particularly interesting because vice versa the level of reducing vitamins in the sprouts is rather low (36). As far as the antioxidant activity is concerned, measured by potassium ferricyanide reduction, the wheat sprout activity (from 1 g of powder) is comparable to that exerted by 1 mg of active pure compounds. As far as the scavenging activity is concerned, the sprout extract activity (from 1 g of powder) is comparable with that of 10 mg of active pure compounds. This indicates that the cocktail of wheat sprout antioxidant molecules is especially powerful on the scavenging of oxygen superoxide. We have reported that the wheat sprout antioxidant activity is able to protect DNA against the oxidative stress induced by Fenton reaction (14). This in agreement with the antimutagenic effect demonstrated with aqueous wheat sprout extracts by Peryt et al. (11, 12) and Tudek et al. (13).

A significant target of our research is now represented by the possibility of obtaining quick separation of large amounts of some classes of wheat sprout antioxidant compounds. In this respect we are trying to separate the polyphenols and glycoside structures of sprout extracts by absorption on charcoal or X-Amberlite resin (Sigma). The results so far obtained show that charcoal is able efficiently to bind the antioxidant compounds containing an aromatic structure but that the subsequent re-elution must be improved. One of the main targets of our present research is to obtain the recovery of large amounts of natural antioxidant substances that could be also employed in the formulation of nutritional complements.

An appreciable goal reached by the experiments described in this paper is the detection of the main antioxidant compounds and the setting up of methodologies to quickly measure the total antioxidant activity exerted by wheat sprout extracts. The suggested analysis may be utilized also to evaluate the differences in activity between different wheat sprout powder preparations.

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