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A comparative study of the antioxidant activity of rice, wheat and spelt

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Summary
In this paper the antioxidant properties of aqueous and hydroalcoholic extracts from seeds and sprout of rice, spelt and wheat, are evaluated. In order to correlate the antioxidant properties with the molecular composition, a preliminary molecular characterization was carried out by several biochemical and spectrophotometric analysis. We have found that the sprouting process strongly increases the antioxidant power, expressed as superoxide radical scavenging activity, reducing power and peroxidase activity. At the same time, the content in molecules soluble in water or ethanol/water (70/30 v/v), included the antioxidant compounds such as polyphenols and flavonoids, seems to be remarkably higher in sprouts than in seeds. In addition we found out that water is more efficient than ethanol/water in extracting active antioxidant compounds. By comparing the three tested cereals extracts, the rice was found to be, at least for what concerns the results here shown, the less active one.

Riassunto
In questo lavoro sono state valutate le proprietà antiossidanti di estratti idroalcolici ed acquosi ottenuti da semi e germogli di riso, farro e grano. Al fine di correlare le proprietà antiossidanti con la composizione molecolare, è stata eseguita una caratterizzazione molecolare preliminare attraverso diverse analisi biochimiche e spettrofotometriche. Abbiamo rilevato che il processo di germogliazione incrementa fortemente la capacità antiossidante, espressa come capacità di cattura dei radicali superossidi, potere riducente e attività perossidasica. Allo stesso tempo, il contenuto di molecole solubili in acqua o etanolo/acqua (70/30 v/v), che include composti antiossidanti come polifenoli e flavonoidi, sembra essere notevolmente più elevato nei germogli rispetto ai semi. Abbiamo evidenziato inoltre che l'acqua è molto più efficiente rispetto al solvente etanolo/acqua nell'estrazione di composti attivi antiossidanti. Mettendo a confronto gli estratti dei tre cereali analizzati, il riso è risultato essere, almeno per quanto riguarda i risultati qui mostrati, il meno attivo.

Introduction

One of the most important messages of modern nutrition is that a diet rich in fruits and vegetables decreases the risk of many diseases including cardiovascular diseases, diabetes, cancer and can even delay age related diseases that are also correlated to oxidative stress (1-10), so an enormous body of research supports the recommendation for people to eat more fruits and vegetables. Many substances, present in fruits and vegetables, are protective: vitamins, polyphenols, carotenoids, thiols, protease inhibitors, saponins, minerals, fiber and so on (11) but often the entire effect is not very likely due to any single nutrient or phytochemical. In fact many researches have shown that the complex mixture of phytochemicals in foods provides protective health benefits more than single phytochemicals through a combination of additive and/or synergistic effects (12).

In the last years, many studies have shown that also cereal grains contain functional constituents that have demonstrated benefits for human health (13-15). Today we know that whole grains are sources of several physiologically active components and health promoter such as dietary fibres, minerals, vitamins and antioxidants. From several years, our research groups are studying cereal sprout and we demonstrated that it contains a cocktail of antioxidant molecules biologically active (16-18).

Why study cereals and cereal sprouts? First of all, cereal crops are mostly grasses cultivated for their edible seeds. Cereal grains provide the most of food energy to the human race. The nutritional relevance of cereals has always been well known and molecules present in the seeds, from which flour is made, are also widely recognized (19).

Recently, several researchers have focused their attention on the extra nutritional properties of cereal seeds and their increase during germination process. In fact, the sprouting not only improves significantly the seed nutritional value but also increases the bioactive molecules content (20). Since from the end of eighties, Peryt et al. (21, 22) and Tudek et al. (23) reported that aqueous extracts from wheat sprouts inhibit the mutagenic effect induced by benzo[a]pyrene in strain TA98 of Salmonella typhimurium and, after oral administration, diminish mice sperm abnormalities inducted by benzo[a]pyrene.

Our research on wheat sprouts has pointed out some peculiarities that we consider very important for healthy nutrition. We have previously demonstrated the presence of many redox enzymes such as catalase and peroxidase in wheat sprouts, which activity appears very strong (16). Considering the possible use of sprouts in the human nutrition, we focused our attention in low molecular weight antioxidant compounds non enzymatically cleaved in the gastro-intestinal tract. We have found the presence of reducing glycosides, polyphenols and thiols from which derives the most of the remarkable antioxidant and radical scavenging activity of wheat sprouts extracts (16). In addition, it has been demonstrated that old mice treated with wheat sprouts extracts showed a recovery of hepatocyte DNA synthesis levels compared to the old untreated ones. Besides, old dogs, affected by senile cataract, orally treated for a month with wheat sprout powder, showed the lens opacity reduced from 25 up to 40% (24).

The aim of this study was to compare the antioxidant activity in seeds and sprouts of different cereals to verify if the germination process is essential to enhance the antioxidant properties not only in wheat but also in rice and spelt and if a comparable antioxidant activity was found in these cereals tested. If the antioxidant activity of rice was comparable to wheat, the coeliac people could benefit from its antioxidant properties using it as functional food.

Materials and methods

Reagents

All reagents were of pure analytical grade. Hypoxanthine, xanthine oxidase from bovine erythrocytes, phosphomolybdic acid, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), Folin & Ciocalteu's phenol reagent, potassium ferricyanide and nitrotetrazolium blue chloride (NBT) and the antioxidant standards (rutin and gallic acid) were obtained from Sigma-Aldrich srl, Milano Italy.
Wheat (*Triticum aestivum*), spelt (*Triticum dicoccum*), rice (*Oryza sativa L.*), from organic agriculture are utilized for all experimental procedures.

Cereal sprout powder

Cereal sprout powders were supplied by Germinal Life (Perugia, Italy) produced as following reported.
The preparation of the sprout powder was carried out taking into account the microbiological safety evaluations and recommendations on sprouted seeds (25). Wheat and spelt 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. Rice was soaked for 14 days in water and the soaking water was changed every 24 hr. After 3-5 days of germination the sprouts were detached as the other cereals. The sprouted cereals were dehydrated at 30 °C and mechanically detached from the seeds by cutter equipped with special blades suitable to achieve the result of detaching the sprout from the seed and of partially breaking it without shattering the seed. The sprouts were separated from the seeds by sieving. The isolated sprouts were then finely ground with a high-speed cutter avoiding the material heating. The sprout powder was preserved in hermetically sealed vacuum vessels.
For the not germinated cereals, we ground the seeds without any other treatment and we utilized them for the extract preparation.
We obtained the following powders derived from these cereals:

1) **W** wheat seeds
2) **S** spelt seeds
3) **R** rice seeds
4) **WS** wheat sprouts
5) **SS** spelt sprouts
6) **RS** rice sprouts

Aqueous (AE) and Hydroalcoholic (HE) Extracts

Extracts from sprout or seed cereal powder were prepared according with the described procedure:
AE: 10 g of each cereal seed or sprout powder were solubilized in 200 ml of water, homogenized in Waring Blendor and then centrifuged at 10,000 x g for 30 min at 4°C. The supernatant was lyophilized and resuspended in water for the following experiments.
HE: 10 g of each cereal seed or sprout powder was solubilized in 200 ml of ethanol/water (70:30 v/v), homogenized in Waring Blendor and then centrifuged at 10,000 x g for 30 min at 4°C. The supernatant was recovered and stored at -20°C overnight and then centrifuged at the same conditions. Following ethanol evaporation at 30°C, the pellet was resuspended in water (recovering of water-soluble molecules), lyophilized and then resuspended in water.
The extract concentration, utilized for each experiment, is specified in the legend to figures.

Reducing power

The total reducing power was measured by utilizing potassium ferricyanide as reagent, following the method of Yen and Chen (26). 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 3000 g for 10

min. The upper layer of solution was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 1:1:2, and the absorbance were measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Superoxide radical scavenging activity

Measurement of superoxide radical scavenging activity was carried out based on the method described by Kirby and Schmidt (27). 20 µl of 15 mM Na_2EDTA in buffer (50 mM KH_2PO_4/KOH, pH 7.4), 50 µl of 0.6 mM NBT in buffer, 30 µl of 3mM hypoxanthine in 50 mM KOH, 5 µl of test samples in water and 145 µl of buffer were mixed in 96-well micro plates (Falcon). 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 40 min using a plate reader (Labsystems Multiskan MS).
Superoxide radical scavenging activity (%) = $(1-A_{sample}/A_{blank})x100$

Thin-layer chromatography (TLC)

TLC was performed on silica gel plates using as solvent system propanol/water 70:30 (v/v). To determine the reducing groups the plates were sprayed with 10% phosphomolybdic acid solution in ethanol (w/v) and heated at 120°C until spot formation was obtained. In fact, phosphomolybdic acid, in the presence of reducing substances, is transformed into molybdenum blue, which is visible on the TLC sheet as a blue spot. To determine the mono and oligosaccharides presence, the plates were sprayed with *p*-anisaldehyde-sulphuric acid reagent. In the presence of sugars brown-grey spots appear. To detect free amino groups, the TLC sheets were sprayed with ninhydrin reagent. In the presence of free NH_2 red-violet spots appear. To determine the phosphate esters, the plates were sprayed with ammonium molybdate-perchloric acid (Hanes reagent). All of the organic phosphate compounds appear as blue spots, whereas inorganic phosphate produces a yellow-green colour (28).

Total phenolic compounds

The amounts of total phenolic compounds were measured according to the Singleton and Rossi method (29) slightly modified. Briefly 20 µl of sample were added to 50 µl of Folin–Ciocalteu reagent. After 4 min, 100 µl of 20% sodium carbonate and 830 µl of water were added. After 2 h of incubation at room temperature, the absorbance at 700 nm was measured. Gallic acid was used for the standard calibration curve (r=0.9995). The results were expressed as gallic acid equivalent/g dry weight of cereals, and calculated as mean value ± SD (*n*=3).

Analysis of flavonoid content

The measuring of the flavonoid content was performed with aluminum chloride reagent, according to Chang et al. (30) slightly modified. To 100 µl of extract was added to make 900 µl, and 60 µl $NaNO_2$ 5% were added. 600 µl $AlCl_3$ 1% were added 5 min later. After 6 min, 400 µl 1M NaOH was added. The solution was mixed well again and the absorbance was measured against a blank at 510 nm. Rutin was used for the standard calibration curve (r=0.9992). The results were expressed as rutin equivalent/g dry weight of cereals and calculated as mean value ± SD (*n*=3).

UV spectrophotometry

The extracts obtained from cereal seeds and sprouts following suitable dilution in water were analysed by UV spectrophotometry from 200 to 400 nm, utilizing a Varian Cary 100 spectrophotometer.

Results

All the experiments have been carried out using aqueous (AE) and ethanolic (HE) cereal extracts.

Extraction yields

Table 1 shows the extraction yields of these two different extraction methods from seeds and sprouts cereal powder. The extraction by water appears more effective than the extraction by ethanol-water (70:30 v/v) and within the same extraction, the yields significantly increase after the germination from about five to ten times depending on the considered cereal.

Antioxidant activity

The antioxidant activity was evaluated by different tests: reducing power, radical scavenging activity and peroxidase activity were measured.

Reducing power

The evaluation of reducing power of a compound is a significant indicator of its potential antioxidant activity. The data, reported in Table 2, demonstrate that a remarkable difference exists between ungerminated and germinated cereals in both extracts. All the seeds have a very low reducing power that increases after germination. The sprouts in fact are from nine to fifty times more active than seeds. Moreover, while the reducing power found in the extracts of the different cereal seeds is pretty much the same, the differences are remarkable in the three examined cereal sprouts, extracted both by water and by 70% ethanol. In the sprout extracts we can point out that: a) EA is much more active of HE; b) the EA reducing power increases from rice to wheat to spelt, while the HE reducing power increases from rice to spelt to wheat.

Superoxide radical scavenging activity

The superoxide radical scavenging activity of the three analyzed cereal seed and sprout AE and HE was determined by using the hypoxanthine-xanthine oxidase system. Not reduced NBT was measured in the absence and in the presence of cereal extracts. The data, reported in Table 2, are quite different from those obtained evaluating the reducing power. In fact, the data here reported present smaller differences compared to those found as reducing power in all examined extract. It is possible to note that the sprout extracts are more active than seed extract and that AE is more active than HE.

It is noteworthy that the differences among the cereals are less evident when extracted in ethanol/water.

Table 1 - Extraction yields of AE and HE from cereal seeds and sprouts. Values are expressed as percentage dry weight of cereal ± SD

	Extract Yield (% Dry Cereals)
Rice Seed HE	1.52 ± 0.05
Rice Sprout HE	10.51 ± 0.81
Rice Seed AE	2.46 ± 0.12
Rice Sprout AE	27.83 ± 3.02
Wheat Seed HE	2.75 ± 0.09
Wheat Sprout HE	18.62 ± 0.71
Wheat Seed AE	5.91 ± 0.21
Wheat Sprout AE	30.46 ± 2.96
Spelt Seed HE	3.15 ± 0.24
Spelt Sprout HE	16.23 ± 1.22
Spelt Seed AE	4.94 ± 0.36
Spelt Sprout AE	22.16 ± 2.19

Peroxidase activity

We previously demonstrated that wheat sprouts (WS) contain remarkable levels of some redox enzymes. In particular catalase and peroxidase activity appears very strong (16).

Here, for what concerns the high molecular weight antioxidant molecules, the presence of peroxidase has been evaluated in AE of cereal seeds and sprouts. The data, reported in Table 2 demonstrate that: a) the strongest peroxidase activity is present in wheat sprout (WS) followed by spelt sprouts (SS) and then by wheat seed (W); b) the peroxidase activity is scanty in the rice seed (R) and sprout (RS) and spelt seed (S). The activity of this enzyme is almost completely absent in HE or in AE after heating at 100°C for 2 hr (data not shown).

TLC analysis

The TLC analysis, carried out with cereal seed and sprout extracts, is reported in Figure 1.

As regards HE (Fig. 1 panel a), in all used staining, we have observed a more evident reaction for sprouts compared to seeds. Phosphomolybdic acid and *p*-anisaldehyde-sulphuric acid reagents show, in the sprouts, the same main spot at RF=0.55 indicating the presence of reducing glycosides in all three cereal sprouts. Also the chromatographic pattern, obtained after ninhydrin staining, indicates a similarity for some molecules present in the sprouts even if the ninhydrin positive spots are more numerous than with the other stainings. Rice sprout shows a stronger reaction to ninhydrin than the other cereal sprouts. If we analyze the TLC of cereal seed HE, we note the presence of further different spots in wheat and spelt when the plates were colored with phosphomolybdic acid and *p*-anisaldehyde-sulphuric acid reagents, while ninhydrin positive spots are quite similar in the three cereal seeds.

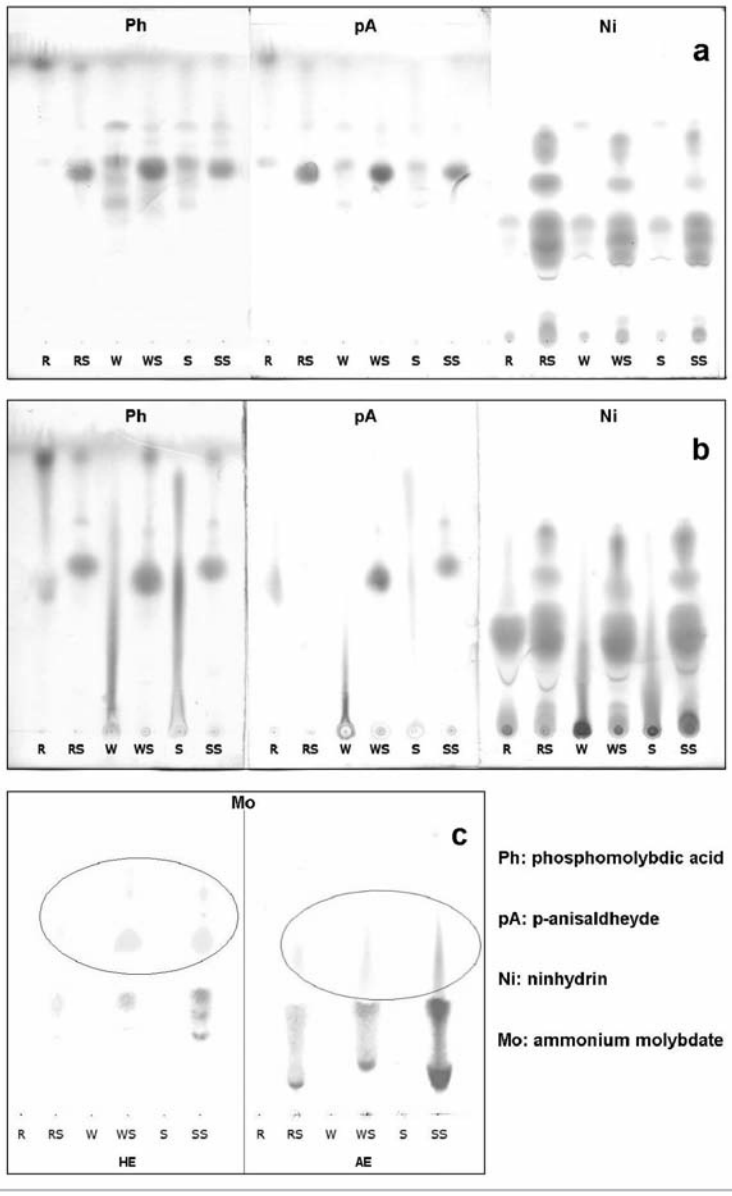
It is interesting to note the lowest presence of reducing groups in rice

Table 2 - Antioxidant activities of cereal seed and sprout extracts

	Reducing Power[a]	Radical Scavenging[b]	Peroxidase Assay[c]
Rice Seed HE	1.93 ± 0.15	0.96 ± 0.15	
Rice Sprout HE	16.56 ± 2.94	3.79 ± 0.08	
Rice Seed AE	1.78 ± 0.07	2.68 ± 0.04	4.34 ± 0.32
Rice Sprout AE	35.01 ± 3.24	5.38 ± 0.31	8.58 ± 0.59
Wheat Seed HE	1.49 ± 0.21	2.15 ± 0.19	
Wheat Sprout HE	31.36 ± 4.42	4.03 ± 0.06	
Wheat Seed AE	1.49 ± 0.25	6.02 ± 0.29	52.46 ± 4.97
Wheat Sprout AE	83.16 ± 5.43	12.4 ± 1.80	165.21 ± 18.34
Spelt Seed HE	1.21 ± 0.19	2.09 ± 0.19	
Spelt Sprout HE	46.92 ± 4.09	3.93 ± 0.17	
Spelt Seed AE	1.9 ± 0.06	4.52 ± 0.22	20.02 ± 1.87
Spelt Sprout AE	69.79 ± 9.27	19.1 ± 2.62	60.56 ± 5.19

[a] Values expressed as μmoles of reduced potassium ferricyanide/g cereal powder ± SD
[b] Values expressed as μoles of not reduced NBT/g cereal powder ± SD
[c] Values expressed as UI/g cereal powder ± SD

Figure 1 - Ascendant TLC chromatography on silica gel plates of HE (panel a) and AE (panel b) of rice, wheat and spelt seeds (R, W, S) and rice, wheat and spelt sprouts (RS, WS, SS). The plates were stained as indicates in the figure. In panel c is shown the phosphorous content in HE (on the left) and AE (on the right). The different extracts are indicated as described before. Yellow spots, corresponding to inorganic phosphorous, are marked by the drawn ellipses. 3 µl of each extract (1 g of cereal powder in 1 ml of water) were seeded



seeds. For what concerns the sprout spot with RF=0.55, we previously reported that, in wheat, probably corresponds to a reducing glycoside that strongly increases during the germination phase of the wheat seeds (1 to 4 days of germination) (31).

In the TLC of the cereal AE (Fig. 1 panel b), the chromatographic pattern shows the same spot pointed out in HE, at RF=0.55, but this analysis is more complex, overall for what concerns wheat and spelt seed. In fact, there is more material which is scantly soluble in the used eluent (propanol/water 70:30). Rice sprout seems to lose completely the p-anisaldehyde positive reaction. A more hydrophobic component, at RF=1, is well evident in phosphomolybdic acid staining, mainly in rice seed. Rice seed shows weaker spots with p-anisaldehyde and phosphomolybdic acid staining compared to the other cereal seeds. Ninhydrin staining shows the presence, in AE, of spots similar to that obtained with HE, but with a stronger reaction.

Also the phosphate content was evaluated by TLC analysis, using Hanes reagent. The results, reported in figure 1 panel c, show that: a) in both extracts used, only sprout extracts are stained; b) the HE are less colored than AE; c) rice HE is the least stained. It is evident, in the TLC, that the

amount of phosphorus increases after germination in all the cereals tested.

Phenolic compounds and flavonoid content

Since a considerable amount of polyphenols is present in wheat sprout extract, we have evaluated the content of polyphenols. The results obtained in five independent experiments are reported in Table 3. Both AE and HE of cereal sprouts contain much more polyphenols than seed extracts. Moreover, spelt and wheat are always the ones with the highest amount of these molecules.
Within the family of reducing polyphenols, the flavonoids may represent an interesting component of the antioxidant compounds synthesized during the germination process, so it has been measured the flavonoid content in the cereal seed and sprout extracts. Flavonoid content is expressed as μg of rutin equivalent per gram of cereal powder and the results are reported in Table 3.
The seed and sprout AE have a higher content of flavonoids if compared to HE and, in particular, spelt sprout AE is the richest in flavonoids.
In contrast with polyphenol content, rice sprout AE and HE have a good content of flavonoids comparable to wheat sprout extracts.

Table 3 - Polyphenol and flavonoid content measured in AE and HE from cereal seeds and sprouts

	Total Phenol Content[a]	Total Flavonoid Content[b]
Rice Seed HE	317.47 ± 25.13	204.26 ± 18.01
Rice Sprout HE	1066.14 ± 70.58	323.38 ± 10.60
Rice Seed AE	407.83 ± 22.19	259.75 ± 16.81
Rice Sprout AE	1982.51 ± 105.39	485.15 ± 32.62
Wheat Seed HE	292.53 ± 15.77	22.69 ± 1.56
Wheat Sprout HE	1523.18 ± 98.08	218.52 ± 14.37
Wheat Seed AE	622.82 ± 48.43	76.71 ± 5.43
Wheat Sprout AE	2793.8 ± 170.44	541.16 ± 27.03
Spelt Seed HE	290.48 ± 24.16	63.95 ± 3.55
Spelt Sprout HE	1969.11 ± 108.68	322.30 ± 13.09
Spelt Seed AE	563.74 ± 29.11	129.06 ± 5.20
Spelt Sprout AE	3086.76 ± 196.65	1219.29 ± 94.12

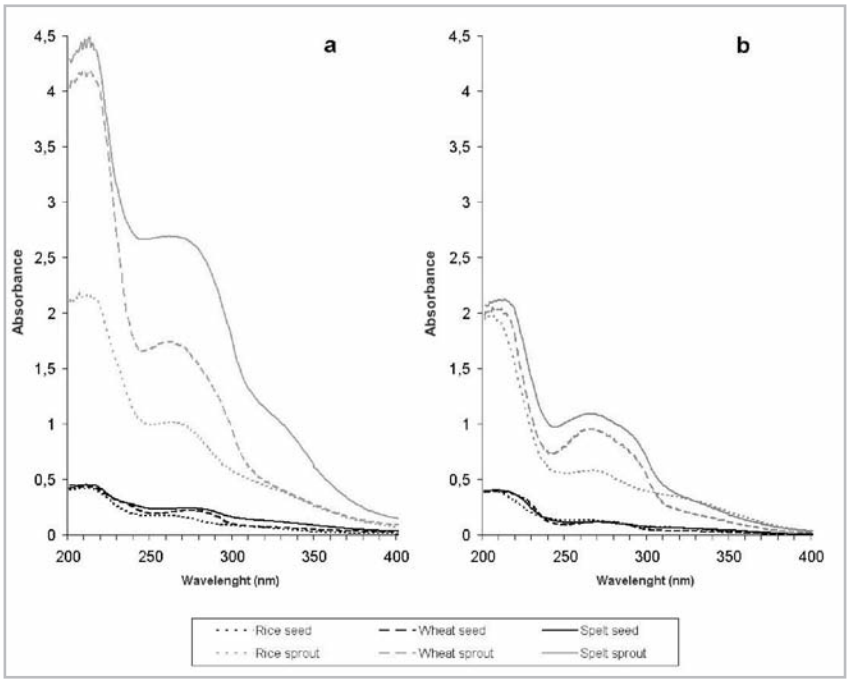
[a] Values expressed as μg of gallic acid equivalent/g cereal powder ± SD
[b] Values expressed as μg of rutin equivalent/g cereal powder ± SD

With regards to seeds, both wheat and spelt HE and AE have a flavonoid content from about five to ten times lower than sprout extracts. Rice seed showes the highest flavonoid content among the cereal seeds tested, but the increase in flavonoids after sprouting is definitely lower (less than two times).

UV spectrophotometric analysis

The analysis of the UV spectra of AE (Fig. 2, panel a) and HE (Fig 2, panel b) indicates some main results: a) the sprout extracts show absorbance levels much higher than seed extracts; b) the spelt sprout extract shows the highest absorbance followed by wheat sprout and rice sprout; c) the AE show UV spectra absorbance at least double than the HE.
In figure 3 is reported the difference spectrum (black line) between the wheat sprout AE and a mixture composed by the aromatic amino acids tyrosine and tryptophan (0.11 mM) and by the nucleotide UTP (0.16 mM), with an absorbance at 260 nm similar to wheat sprout AE.
The difference spectrum shows two main peaks: the 300 nm peak may be representative of polyphenolic compounds, the 230 nm peak is probably due to polymerisation bonds, for example peptidic bonds or glycosidic bonds. Very

Figure 2 - Absorption spectra from 200 to 400 nm of AE (panel a) and HE (panel b). All the extracts (1 g of cereal powder in 1 ml of water) were diluted 1:200 in water for spectrophotometric analysis



similar results were obtained with the other AE and HE sprouts extracts (data not shown).

Discussion

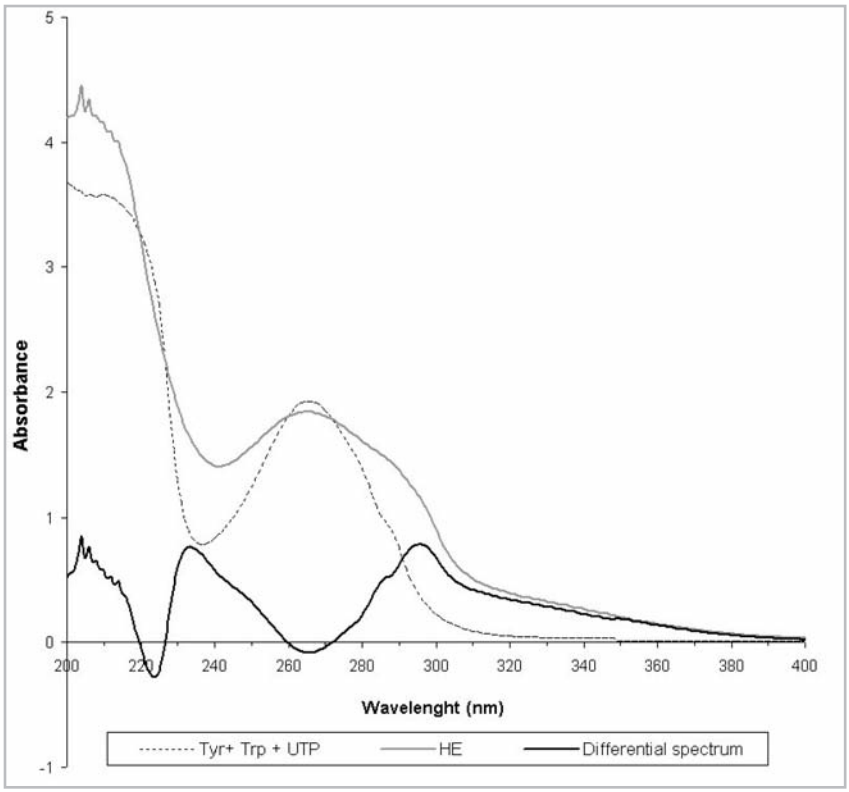
In this paper we have demonstrated that, in all cereals tested, the antioxidant activity increases with germination process. The cereal sprouting, in fact, induces not only the already known morpho-physiological variations, but also a strong increase of phosphates and antioxidant compounds content, such as polyphenols and flavonoids and this is reflected in their antioxidant activity. Furthermore, there are remarkable differences, not only before and after sprouting, but also within the three considered cereals. In fact, each activity or biological molecule, here tested, seems to be lower in rice in comparison with wheat and spelt that, instead, seem to have similar properties.

In our previous papers we demonstrated that wheat sprouts show: a) a very strong activity of many kinases (for example CKII kinase); b) particularly high levels of organic phosphates; c) a strong antioxidant activity due to a powerful cocktail of antioxidant molecules; d) an antioxidant activity notably higher than that of wheat germ and wheat young plant; e) remarkable levels of some redox enzymes (in particular catalase and peroxidase); f) a prominent fraction, at low MW, probably represented by reducing glycosides (16, 18, 30).

The observed differences between ungerminated and germinated cereals are shown by TLC analysis, evaluation of phenolic and flavonoid content, peroxidase activity, UV spectra analysis.

Previously it has been demonstrated that, for what concerns the phosphate content, the major phosphorus containing molecule in the seed is phytin. During wheat germination it is necessary, for the cereal seed embryo, to be supplied with inorganic phosphate which is enzymatically liberate from phytin in the aleurone layer and it may be regulated by phytase (32). Accordingly, our results demonstrate the presence of inorganic and mainly organic phosphorus only in germinated cereal seeds. In fact, since the phytin is present, in cereal seeds, as inorganic insoluble Ca/Mg salt, the extraction method doesn't allow to recover this molecule. The organic phosphate increase, in cereal sprouts, agrees with the increase of kinase activity previously demonstrated in the wheat sprout extract; in fact the

Figure 3 - Absorption spectra from 200 to 400 nm of wheat sprout HE (1 g of cereal powder in 1 ml of water diluted 1:200), of Trp, Tyr, UTP mixture and their differential spectrum



phosphorylation activity by endogenous kinases is from 10 to 40 time higher in wheat sprouts than in wheat seeds (24). This could support the results obtained also with other cereals (33).

If we consider, for the antioxidant enzymes, the peroxidase activity, we note that it is particularly abundant in wheat sprout and this is, perhaps, the strongest difference between wheat and spelt sprouts.

The differences found in the antioxidant content and activity in the three cereal ungerminated and germinated seeds, are confirmed by UV spectra.

The UV spectrophotometry demonstrates that the germination process causes a dramatic increase of the absorbance in the range 250-350 nm. Taking into account that the sprouts are constituted of fast growing cells, the presence of significant amounts of nucleotides and aromatic amino acids must be considered. For this reason we performed the UV difference spectrum between the cereal extracts and a mixture of the aromatic aminoacids and UTP. The cereal sprout extracts could contain nucleotides and amino acids but even if we assume, *ab absurdo*, that the absorbance at 260 nm is completely due to these molecules, the absorbance at 300 nm demonstrates the presence of remarkable levels of compounds which may be referred to polyphenols. So the absorbance ratio 300nm/260nm should be proportional to the polyphenols presence. The absorbance ratios 300nm/260nm of the AE of spelt, wheat and rice (0.64, 0.53, 0.58, respectively) and of the HE of spelt, wheat and rice (0.63, 0.55, 0.68, respectively) indicate that the mixtures of compounds in the various extracts are qualitatively similar. In addition, the UV spectra show that spelt sprout extract contains more material than wheat sprout extract and wheat sprout extract contains more material than rice sprout extract. Moreover, the extraction by water appears more effective than the extraction by ethanol-water (70:30 v/v). It is noteworthy that the absorbance values are quite similar to the data obtained by titrating directly the content of polyphenols.

As far as it concerns the recovery of antioxidants from aqueous and hydroalcoholic extracts, it could be surprising that the extraction by water gives a yield of active mole-

cules remarkably higher than the extraction by ethanol-water (70:30 v/v). In fact the antioxidant compounds often contain in their structure a partially hydrophobic moiety. However, the extraction by ethanol-water causes the precipitation of many macromolecules, so the co-precipitation of a significant portion of antioxidant polyphenols may be hypothesized. This taking also into account that the presence of 70% ethanol lowers the dielectric constant with a proportional increase of the intermolecular interaction strength. Accordingly to what already stated, the re-extraction by water of the pellet obtained following the wheat sprouts extraction by ethanol/water gives a further recovery of antioxidant compounds of about 34%. Vice versa, the re-extraction by water of the pellet obtained following the wheat sprouts extraction by water is remarkably lower (about 15%, data not shown). A better recovery of polyphenols and an increase in antioxidant activity was also reported by Fernandez-Orozco et al. (20).

From all the experiments reported, we can conclude that the AE is always more active than HE. This could be referred to the higher extraction yield, but since also the seed extraction yield is higher in AE, without a significant increase of activity, this cannot be the main reason.

In fact only in the sprouts the increase of biological activity corresponds to an increase of extraction yield. So, in our opinion, different molecules, present only in sprouts, contribute to antioxidant effect.

Another noteworthy result, is that rice (neither ungerminated nor germinated), a gluten-free cereal, seems, at least for what concerns the results here shown, less active respect to spelt and wheat. However, the shown antioxidant properties may justify its use as functional food addressed to coeliac subjects.

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