SPROUTING AS A METHOD OF INCREASING THE TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF SELECTED SEEDS

GERMINAREA SEMINȚELOR – METODĂ DE MAJORARE A TOTALULUI FENOLIC ȘI A CAPACITĂȚII ANTIOXIDANTE

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Rezumat. Scopul acestui studiu este de a investiga efectul germinării asupra totalului fenolic și acțiunii antioxidante a semințelor de grîu, floarea soarelui și porumb. Germinarea majorează totalul fenolic la fel ca și capacitatea antioxidantă, evaluată utilizînd metoda DPPH. Printre probele cercetare, semințele de grîu se deosebesc prin valorile cele mai scăzute ale totalului fenolic și capacității antioxidante. Germenii de porumb și floarea soarelui sunt cei mai bogați în conținutul de fenoli și manifesta cea mai înaltă capacitate antioxidantă. În această lucrare s-a dezvăluit că germenii sunt o sursă reușită de compuși bioactivi cu antioxidanți în rația noastră alimentară.
Cuvinte cheie: Germeni, fenoli, antioxidanţi, compuşi bioactivi.

Abstract. The aim of this study was to investigate the effect of germination on the phenolic content and antioxidant activity in the seeds of wheat, sunflower and maize. Germination increased total phenolic content as well as antioxidant activity, evaluated using DPPH method. Among the samples, wheat seed was characterized by lowest levels of total phenols and antioxidant activity. Maize and sunflower sprouts were the most rich in phenols and exhibited the highest antioxidant activity. In this work it was found that the sprouts are a good source of bioactive compounds in our diet with health-promoting antioxidants.
Keywords: Sprouts, phenols, antioxidants, bioactive compounds.

Introduction

The role of agriculture in terms of employment, exports and poverty reduction in Moldova's economy is indisputable. An ingenious question towards the occupation that constitutes more than 16.2% of GDP and involves 40.6% of population is its role in adequate health maintenance and the perspectives of pharmacotherapeutical use of the products.

Zea mays, Triticum aestivum, and Helianthus annuus are our national cultures that in the last 3 years record slow but certain increase in cultivated surface and amount of harvest. They are named staple food because of their economic availability, high nutrient value and balance, ability to face world population growth; as well they are basic ingredients of bread and other bakery products [1]. Currently there is an up-to-date preference among consumers for food systems that contain not only traditional nutrients but also provide other compounds that are beneficial to health and well-being. Grains are rich in polysaccharides (starch – between 60% and 75% of the total dry weight), fibers (soluble ones which include pectic substances and hydrocolloids and insoluble type made of cellulose, hemicellulose and lignin) proteins (albumins, globulins and the gluten complex- glutenins and gliadins – proteins that will form the gluten at dough making), lipids, vitamins (essential in human genetic regulation and genomic stability: folic acid, vitamin B12, vitamin B6, niacin, vitamin C, vitamin E and D), minerals (Fe, Zn, Se) [2].

Meanwhile seeds contain significant amounts of undesirable compounds, such as oxalic acid, lectins and phytic acid, one the most known antinutrients, located in the germ and acting as a defense mechanism. Most of the inorganic phosphorus (Pi) present in mature cereal seeds (40–80%) is stored as phytate that forms complexes with minerals such as Ca^{2+}, Fe^{3+}, Zn^{2+} and Mg^{2+} reducing their bioavailability. It was proved that soaking does not reduce phytic acid at all in grains that have been heat-treated, because they no longer contain the phytic acid-degrading enzyme phytase [3]. Cooking without soaking first also hasn't effect on phytic acid. Anyway more than 50% of world daily caloric intake is derived directly from cereal grain consumption. And we should be aware of cruel disadvantages of whole grains that limit their use: they are loaded with gluten, spike blood sugar rapidly, contains substances that "steal" nutrients from the body, are associated with several brain diseases (cerebellar ataxia, schizophrenia), may be addictive, drastically raises LDL cholesterol. More than 55 diseases have been linked to gluten. It's estimated that 99% of the people who have either gluten intolerance or celiac disease are never diagnosed [4].

Phenolic compounds, a group of above 8,000 phytochemicals that received considerable attention for being potentially protective factors against degenerative diseases, are the most widely spread class of secondary metabolites in our day by day diet, their total intake constitutes approximately 1 g/d, 10 times higher than dietary vitamin C and 100 times higher than vitamin E and carotenoid levels. In food polyphenols contribute to the bitterness, astringency, color, flavor, odor and oxidative stability. Their health effects depend on their respective intake, synergism and bioavailability [5]. These ubiquitous chemical compounds are centered on response to pathogen attacks, UV protection, cytotoxic antitumor activity and the most cited one – the antioxidant activity. Pollution, cigarette smoke, drugs, stress increase free radical exposure. Even the initial attack of these electrically charged molecules is neutralized, because of the unpaired electron a chain reaction occurs. The cells most frequently damaged by oxidative stress are unsaturated fatty acids in lipids, cholesterol, different functional polypeptides and proteins, and nucleic acids. That's why free radicals lead to cumulative and debilitating diseases like diabetic retinopathy, Parkinson's and Alzheimer's diseases, adult respiratory distress syndrome, ischemic arrhythmia, kidney failure, AIDS, diabetes, etc. Mechanisms of antioxidants consist of free radical quenching, transition metal chelating, reducing peroxide, and simulation of in vivo antioxidative enzyme activities [6]. Polyphenols have a perfect antioxidant structure, due to the presence of an aromatic phenolic ring that can stabilize the unpaired electron. The phenolic groups in polyphenols accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components. So, among vitamin C, vitamin E and carotenoids they protect the body's tissues against oxidative stress.

Materials and methods

The seeds of wheat, maize and sunflower were harvested in the village Rădeni, district Strășeni in 2015. For obtaining the dry extract an amount of 20.0 g was weighed, grinded using a mortar and a pestle, added a quantity of 96% ethanol, mixed on a high speed, extracted nearly 24 h. The extraction was performed 3 times consecutively till depletion and finally introduced in a rotary evaporator.

Total phenolic content of extracts was measured by employing the Folin–Ciocalteu assay [7]. An aliquot of 50 µl of an extract was mixed with 250 µl of Folin–Ciocalteu phenol reagent (10 x diluted), 500 µl water, and allowed to react for 1 min. Then 800 µl of Na_2CO_3 solution 20 % was added and allowed to stand for 2 h (30 min at 40 ˚C) before the absorbance of the reaction mixture was read at 760 nm against a blank without extract. The total phenolic content of the extracts was expressed as mg gallic acid per gram of plant material on dry basis.

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of the free radical – scavenging activity of the extracts [8]. It is a free radical at room temperature which produces violet color in methanol and it is reduced in the presence of an antioxidant molecule, giving rise to uncolored solution. The use of DPPH provides an easy and rapid way to evaluate antioxidants. Sample stock solutions (1 mg/ml) were diluted to final concentration of 200, 100, 50, 25, 10, 5 and 1 µl/ml in ethanol. Different concentrations of each extract were added, at an equal volume (0.75 ml), to ethanolic solution of DPPH (1.5 ml, 20 mg/l). After 15 min at room temperature, the absorbance was recorded at 517 nm. Ethanol was used as the blank. DPPH solution (1.5 ml, 20 mg/l) and ethanol (0.75 ml) was used as the negative control. The IC_{50} value was calculated graphically and it denotes the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Results and discussions

From the results it was revealed that sprouts exhibited greater total phenolics than seed extracts in terms of gallic acid equivalent (Figure 1). The sprouts and seeds of *Zea mays* showed 48.44±0.82 and 20.87±0.31 mg GAE/g dried weight respectively. Similarly the sprouts and seeds of *Triticum aestivum* showed 22.05±1.49 and 17.35±0.47 mg GAE/g dried weight respectively while, sprouts and seeds of *Helianthus annuus* showed 86.14±0.47 and 52.96±3.16 mg GAE/g dried weight.

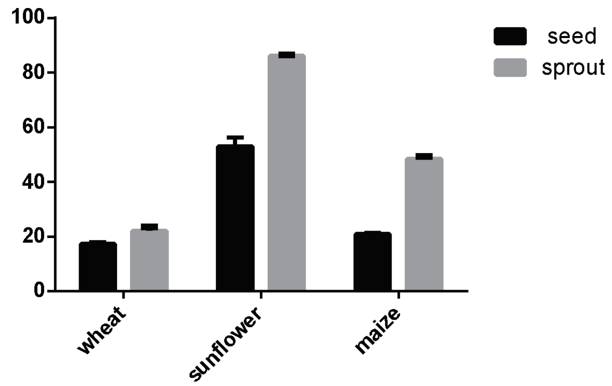

Figure 1. Total phenol as gallic acid equivalent (mg GAE/g dried weight)

In all assays, the extracts of sprouts showed the highest values of antioxidant activity as shown in the Table 1. The results revealed that the IC_{50} value was found to be the highest in sprouts of *Helianthus annuus* and lowest in seeds of *Triticum aestivum*. On the basis of the results of DPPH radical scavenging activity for IC_{50}, the plants were placed in the following order: sprouted *Helianthus annuus* > sprouted *Zea mays* > seed *Zea mays* >seed *Helianthus annuus* > sprouted *Triticum aestivum* > seed *Triticum aestivum*. The evident correlation between the two experiments is the major increase of total phenolic content and antioxidant capacity in sprouts in relation to seeds.

Table 1. **Free radical scavenging ability of the seeds and sprouts by the DPPH method[1,2]**

	DPPH IC_{50} (μg/ml)	
	Seeds	***Sprouts***
Wheat	766.26 ± 2.32[a]	653.21 ± 1.15[a]
Sunflower	428.21 ±2.54[b]	**128.81 ± 1.68[b]**
Maize	347 ± 2.03[c]	169 ± 0.63[b]
Ascorbic acid	0.60 ± 0.01	
Gallic acid	1.50 ± 0.02	

[1]The values are mean ± standard deviation

[2]Values in the same column not sharing the same superscript were significantly different from each other ($p < 0.05$)

Conclusions

Experimentally it was revealed that sprouts exhibited higher total antioxidant capacity compared to seed extracts. The results showed that sprouts are a promising source of phenolic compounds with antioxidant potential.

Sprouted grains extract could be used in food as an additive, i.e. as a source of natural antioxidants in order to replace the synthetic ones. Thus, sprouted cereals, due to easy availability, can serve as good substrates offering significantly low-cost, nutritional dietary supplements and bioactive compounds, and had a tremendous potential in food and pharmaceutical industry.

Sprouting may be suggested as an alternative for increasing the total phenolic content and the antioxidant capacity of the seeds, as a quick, accessible, accurate, non-destructive and safe (lack of side effects) method.

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