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SPROUTED BUCKWHEAT AN IMPORTANT VEGETABLE SOURCE OF ANTIOXIDANTS

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Germination process is the only food processing which provides a significant increase of the nutritional value by enhancing the bioavailability of some nutritional compounds, such as vitamins. The aim of our study was to test the antioxidant properties of the buckwheat sprout. The total polyphenols, flavonoids and ascorbic acid contents during seven days of germination were determined. The results indicate that important changes occur in the amount of biologically active compounds during germination: the amount of polyphenols increases from 50.26 to 298.03 mg/100g d.w., the amount of rutin increases from 13.66 to 283.43 mg/100g d.w., the amount of quercitin increases from 4.77 to 223.76 mg/100g d.w., whereas the amount of ascorbic acid increases from 0 to 1.09 mg/100g d.w. Due to the excellent antioxidant properties, the buckwheat sprouts can be considered good candidate ingredients for functional foods to be used for lowering the risk of various diseases and/or for exerting health promoting effects in addition to its nutritive value.

Keywords: buckwheat, buckwheat sprouts, germination, antioxidants, rutin, flavonoids, ascorbic acid

Introduction

Buckwheat (*Fagopyrum esculentum Moench*) is a pseudocereal that belongs to the *Polygonaceae* family (Tanaka *et al.*, 2002). It has been used both in food formulation and as traditional medicine (Marshall *et al.*, 1982). Buckwheat is rich in nutrients, such as protein, aminoacids and mineral compounds (Ikeda, Yamashita and Murakami, 1995).
For many years, the cultivation of buckwheat was in decline in Romania, Denmark, Germany, France, Moldova, and U.S.A. Due to its health-promoting properties, recently the buckwheat cultivation increased in France and U.S.A.

Germination improves the nutritive value of cereals and has been found to decrease the levels of anti-nutrients compounds present in cereal, therefore maximizing the levels of utilizable nutrients (Mohamed *et al.*, 2007; Inyang and Zakari, 2008).
Germination was suggested to be a suitable technological procedure for improving the nutritional quality of cereals and other seeds (Gulewicz *et al.*, 2008). This is a consequence of enzymes activation and their involvement in the synthesis of a wide range of chemical compounds causing the enhancement of nutritional quality (Taraseviciene *et al.*, 2009). Germinated seeds are rich in vitamins, minerals and are also reported to contain phytochemicals important for disease prevention (Fernandez-Orozco *et al.*, 2006). An increase in the bioavailability of minerals and vitamins has been observed due to germination (Sulieman *et al.*, 2007). In addition, germination is a simple tool that allows enhancing the palatability and digestibility.
The antioxidant compounds from foods play important roles as health protecting factors. A variety of biological functions, e.g. anti-mutagenic, anti-carcinogenic, and anti-aging, originate from that property (Holasova *et al.* 2002).
Buckwheat seeds contain several components with healing benefits, such as flavonoids and flavones, phenolic acids, phytosterols, fagopyrins and thiamin-binding proteins.
Flavonoids represent a major group of natural antioxidants. Epidemiological studies have suggested a protective role of dietary flavonoids against coronary heart diseases and possibly cancer (Chao *et al.*, 2002; Hertog *et al.*, 1995). In recent years, flavonoids have attracted increasing interests due to their various beneficial pharmacological effects including anti-allergic, anti-viral, anti-cancer and antioxidant properties (Chao *et al.*, 2002; Fotis *et al.*, 1997). Flavonoids are known for their effectiveness in reducing cholesterol levels in blood, keeping the wall of capillaries and arteries strong and flexible, reducing high blood pressure and reducing the risk of arteriosclerosis (Fabjan *et al.*, 2003; Li & Zhang, 2001). Six flavonoids have been isolated from buckwheat grains. Of the total pool of buckwheat grains flavonoids, rutin was observed to predominate (Kreft *et al.*, 1999). Rutin, quercitin, orientin, vitexin, isovitexin, and isoorientin were identified in buckwheat hulls (Dietrych-Szostak & Oleszek, 2001). Quercitin (3,4-di-hidroxy-flavonol) is the most intensively studied flavonoid due to its effective antioxidant activity and significant absorption in gastro-intestinal tract. It exists predominantly in glycosylated forms such as rutin (quercitin -3-o-beta-rutinoside). Rutin is used medicinally in many countries to reduce capillary fragility associated with some hemorrhagic diseases or hypertension in humans (Yildzogle-Ai *et al.*, 1991).
Polyphenols are secondary plant metabolites that play a role in the protection of plants against ultraviolet radiation, pathogens and herbivores (Harborne & Williams, 2000). Several hundred molecules having polyphenol structure have been identified in edible plants (Manach *et al.*, 2004).
Ascorbic acid is an essential nutrient of life, involved in the production of glucocorticosteroids and certain neurotransmitters, which facilitates the absorption of iron in the digestive system. Ascorbic acid is well known as a powerful antioxidant, protecting against oxidative damage to DNA, membrane lipids and

proteins. It does not exist in buckwheat seeds, but is synthesized during germination.
Nutritionists recommend eating many foods rich in antioxidants, thus, maximizing the potential of natural antioxidants has become a priority in obtaining functional food.
Functional foods are considered those foods which are intended to be consumed as part of the normal diet because they contain biologically active components and have potential in reducing the risk of disease.
Germination is the only process of agro-food processing which provides significant increase of the nutritional value by increasing the bioavailability of vitamins and bioelements and other biologically active compounds.
The aim of our study was to investigate the evolution of the antioxidant potential of buckwheat during germination.

Materials and methods

Materials

Commercial buckwheat (*Fagopyrum esculentum Moench*) retailed on the local market (Bucharest, Romania) was used in this study.
Buckwheat seeds were germinated in the EasyGreen germinator, which keeps the constant humidity by water-spraying at regular intervals. Seeds were germinated for seven days and samples were taken every 24 hours. After germination the seeds were dried at 40°C and used for the estimation of antioxidants and ascorbic acid content.
Buckwheat seeds were washed very well and afterwards were subjected to germination according to Figure 1. After 7 days of germination, the samples were dried at 45°C, and then ground in a ball mill.

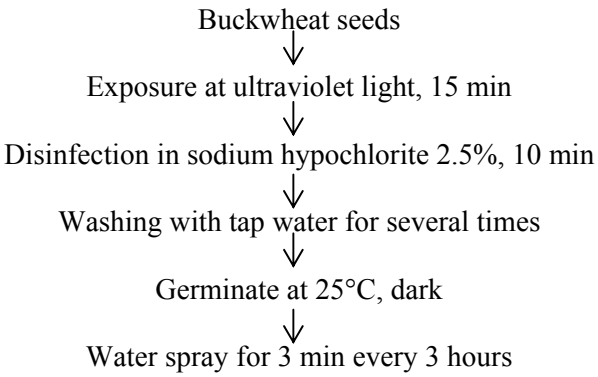

Figure 1. Schematic representation of buckwheat processing

Methods

Extracts preparation

The extraction (1:20) was made using ethanol 70% aqueous solution, for 2 hours on an ultrasounds stirrer. Samples were afterwards filtered and the filtrate was used for determination of polyphenols, flavonoids and ascorbic acid contents.

Total polyphenols content

Total polyphenols (TP) contents were determined as described by Waterhouse (2002). Sample extracts were prepared by diluting 1:10. To proceed with the Folin-Ciocalteu method, 1 ml of sample extract was mixed with 5 ml of Folin-Ciocalteu reagent and 4 ml sodium carbonate solution 7.5%. The blank was prepared using the same chemical reagents without the sample extract. The mixtures were well homogenized and left in the dark, at room temperature (25°C) for 60 minutes, then the absorbance was read at λ=765 nm (Mahnaz, 2009). UV/VIS spectrophotometer and 1 cm quartz cells were used for all absorbance measurements. The concentrations are expressed as mg of gallic acid equivalents per 100 g of dry weight.

Total flavonoids content

Total flavonoids content was measured by aluminum chloride colorimetric assay with modification. To the extract solution (1 ml) or standard solution of rutin or quercitin (0-50 µg/ml) was added 1ml aluminum chloride (2.5%), 2ml sodium acetate (10%) and ethanol 70% up to 10 ml. The blank was prepared using the same chemical reagents excluding the extract. The flasks were mixed well and left in the dark, at room temperature (25°C) for 30 minutes, then the absorbance was read at λ = 420 nm. UV/VIS spectrophotometer and 1 cm quartz cells were used for all absorbance measurements. The concentrations are expressed as mg of rutin or quercitin equivalents per 100 g of dry weight.

Ascorbic acid content

Ascorbic acid content was measured by iodometric method according Vata *et al.* (1992).

Statistical analysis

All experiments were carried out in triplicates. Statistical analysis was done using Microsoft Excel 2007. The average values are reported together with standard deviation.

Results and discussion

Evaluation of total phenolic content

Phenolics are very important constituent of plants. Their free radical scavenging ability is attributed to hydroxyl groups. Total phenolics were measured using an established method employing the Folin-Ciocalteu reagent. The principle of this method is the reduction ability of the phenol functional group.

The evolution of the amount of total phenolic compounds in buckwheat during seven days of germination is presented in Figure 2.
We can see that phenolic compounds content is influenced by germination time and varied between 50.26 mg/100 g d.w. in buckwheat seed and 298.03 mg/100 g d.w. in buckwheat sprouts in the seventh day after germination. The increase of phenolic compounds in the germinated buckwheat can be explained by an increase in the amount of free forms occurring as a consequence of hydrolytic enzyme activity, due to the breakdown of the cell wall, during germination. The relatively high amounts of polyphenolic compounds in sprouted buckwheat give reasons for continuing the research as planned by estimating the antioxidant activity.

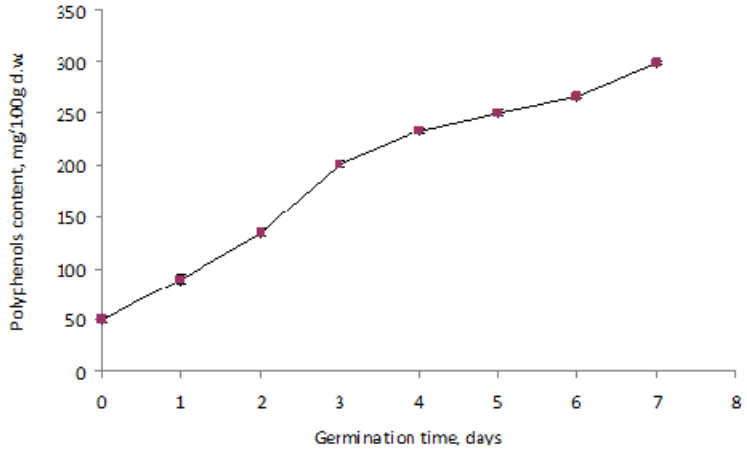

Figure 2. Evolution of polyphenols during buckwheat germination

Evaluation of flavonoids content

The evolution of flavonoids, expressed as rutin and quercitin, during seven days of the germination step is presented in Figure 3.

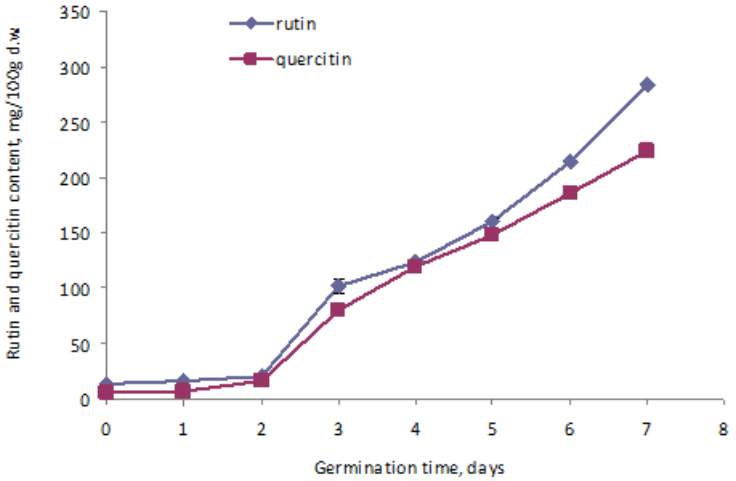

Figure 3. Evolution of flavonoids during buckwheat germination

The rutin content increased from 13.66 mg/100 g d.w. extract in buckwheat seed to 283.43 mg/100 g d.w. extract in buckwheat sprouts in the seventh day of germination.
The quercitin content increased from 4.77 mg/100 g d.w. extract in buckwheat seed to 223.76 mg/100 g d.w. extract in buckwheat sprouts in the seventh day of germination.
Buckwheat seeds contain small quantities of rutin and quercitin. As germination days progressed, the contents of rutin and quercitin were gradually increased. From these results, the sprouted buckwheat is considered as a good dietary source of rutin and a new functional food.

Evaluation of ascorbic acid content

Ascorbic acid increased rapidly with prolonging the germination time (Figure 4).

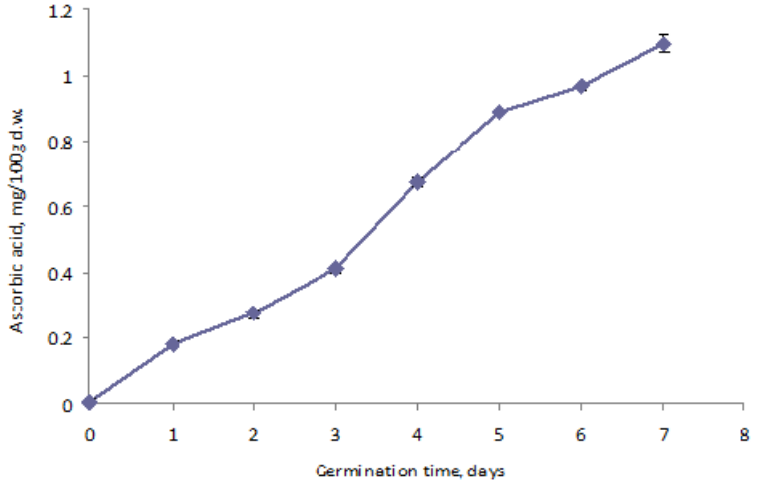

Figure 4. Evolution of ascorbic acid during buckwheat germination

During germination process, ascorbic acid content had an interesting evolution. In buckwheat seed, the water-soluble ascorbic acid does not exist. But as seeding progressed, the content of ascorbic acid increased rapidly; in the seventh day of germination, it reached a maximum value of 1.09 mg/100 g d.w.
Dry seeds are devoid of the ascorbate reduced form (ASC) and contain only dehydroascorbic acid (DHA) and ASC peroxidase (Klapheck *et al.*, 1990; Tommasi, 1999). During buckwheat germination, remarkable changes in the content and redox balance of ascorbate occured both in the embryos and in the endosperm. The absence of ASC in the dry seeds and its progressive increase during germination is not a peculiarity of Fagopyrum esculentum, since the same behaviour has been observed in some angiosperm seed. According to data reported here, the increase in ASC and the change in the ASC/DHA ratio occurring during germination are probably due to ex novo ASC biosynthesis rather than the ASC recycling capability.

Conclusions

The antioxidant potential of buckwheat was monitored during seven days of germination at 25°C. Our results indicate that the amounts of total polyphenols, rutin, quercitin and ascorbic acid increase during the whole period of sprouting.
The sprouted buckwheat with enhanced functionality can be afterwards processed through drying. The buckwheat flour is the basis for obtaining a wide range of functional foods with positive impact on consumers.

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