

Impact of germination on phenolic compounds content and antioxidant activity of alfalfa seeds (*Medicago sativa* L.)

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Received: 26 February 2013; Accepted: 17 March 2013

Abstract

Phenols have been widely studied and confirms to posses diverse bioactivities which could be beneficial to human health. Alfalfa seeds (*Medicago sativa* L.), but also the soy beans (*Glycine max* L.) which will be part of this study, belong to the Fabaceae family, generally known by their often edible seeds. Nutritional properties of Fabaceae have been investigated for a long time and the legumes have been shown to have many beneficial health effects. In addition to this two plants, clary sage (*Salvia sclarea*) is another important source of health-promoting phytochemicals. Flavonoids are polyphenolic compounds with potential beneficial effects on the menopausal symptoms.

The object of the present research is to analyze polyphenolic compounds and antioxidant activity of alfalfa sprouts, after a day until seven days of germination. A hydroalcoholic extract and ultrasound assisted extraction performed a good polyphenolic compounds and a higher antioxidant activity level in the second, third, sixth and seventh day of germination. In this study it was found that the sprouts are a good source of bioactive compounds in our diet with health-promoting antioxidants.

Keywords: Alfalfa sprouts, polyphenols, antioxidant activity

1. Introduction

Extensive research has been performed worldwide and important evidences were collected to show the immense potential of plants used in various traditional therapeutic systems.

So, it is well known that plant-based diets rich in phytochemicals, primarily with antioxidant properties, can decrease the incidence of cardiovascular diseases and several types of cancer [1]. The benefits towards many of these conditions come in part through the antioxidant characteristic of phenols; therefore, it is important to quantify, identify and evaluate their antioxidant activities. Due to the potential significance of phenolic antioxidants for the prevention of a wide range of degenerative physiological processes, it is necessary to identify plant sources with optimum physiological stages for maximising phenolic accumulation.

Little is known about variations in phenolic concentrations during seed germination [2]. The common name of the herb Alfalfa (*Medicago sativa* L.) is Lucerne. In folk medicine, this herb is used in alternative herbal treatments. The medicinal value of the plants lies in their phytochemical components which produce definite physiological actions in the organism. The most important bioactive components are starch, carbohydrates, basic proteins (histones and L-lysine, L-arginine, aspartic and glutamic acids) and the non-protein amino acid (L-canaverine). Alfalfa has high contents in tannins, pectin substances, saponines, amines, coumarin derivatives, triterpene glycosides, carotenoids, purines base, plant sterols, phytoestrogens (cumestrol), flavones, isoflavonoids and phenolic compounds [3].

It is a remarkable source of vitamins A, D, E, and K. *Medicago sativa* belongs to the *Leguminosae* family; it is called the "father of all plants" and is considered the green food of the millennium. An important

quality of alfalfa is the strengthening of the immunity. The ingredients of the alfalfa plant are used fresh, in order to maintain the essential nutrients necessary for proper functioning of the whole body [4,5]. Sprouts of alfalfa have medicinal use in many metabolic deficiencies, are phytonutrient-rich, provide significant amounts of antioxidants [6,7,8], delay the aging processes, beneficial effects on the menopausal symptoms, help to strengthen the immune system, especially protect against infection, prevent heart disease and coronary heart disease (through decreasing plasma cholesterol) [9,10].

In recent years, the consumption of sprouts has been growing worldwide, because they are often perceived as part of a healthy diet [11]. Regardless of the place and season, sprouts can easily be grown in a short period, the price is low and are usually eaten from the age of 5 to 7 days.

Sprouts are believed to be rich in health-promoting phytochemicals compared with their mature counterparts. Germination (sprouting) has been suggested as an inexpensive and effective way to improve the quality of legumes. Sprouting mobilizes polymerized forms, such as concentrated starch and protein, into carbohydrates and free amino acids, respectively. This significantly improves the nutritional value of sprouts, which can be readily used by the human body [12]. Thus, germination can lead to the development of such functional foods that have a positive effect on the human organism and that help in maintaining the health [13].

The objective of this study is to identify germination stage where the level of phenolic antioxidants is high and release edible sprouts with bioactive compounds for nutraceutical properties. In the present study, we have characterised the levels of polyphenolic compounds and antioxidant activity for alfalfa sprouts during the 7 days of germination.

In addition to this work, sprouting alfalfa seeds, will come another two important source of health-promoting phytochemicals, soy beans (*Glycine max L.*) and clary sage (*Salvia sclarea*), also a very gut source of bioactive compounds which are helpful in maintaining healthy women aged 50-55 years old.

2. Materials and Methods

2.1. Materials. Organic alfalfa seeds (*Medicago sativa L.*) - Country of origin Canada - purchased from health stores, Germinator 4 polystyrene dishes – Kanud, Drying device (38,9°C), DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium carbonate and Folin–Ciocalteu reagent, polyphenolic standards.

2.2. Seed germination. Seeds were sterilised with an UV Lamp for 1h and after disinfection, seeds were allowed to imbibe water for 6h at 20°C. Then water was removed and seeds were dark germinated in the Kanud germinator at 25°C, after germination the sprouts were dried at 38,9°C. This method of drying was chosen for maximum effectiveness in preserving bioactive compounds. A hydroalcoholic extract was prepared using food grade ethanol, 99.8% and deionised water. Ultrasound assisted extraction was performed in an ultrasound cleaning bath for 60 min.

2.3. Methods. Moisture content according to the AOAC - 1995 method, determination of polyphenols by Folin Ciocalteu method, determination of flavonoids by spectrophotometric method, determination of antioxidant activity by DPPH method was performed.

3. Results and discussion

Drying was carried out in accordance with the moisture content of the sprouts, which has a duration of 4h until 72h. The sprouts at day 4 have 14,31% humidity and is the followed dry matter content on drying and is represented in the Figure 1.

The alfalfa sprouts are a good source of bioaccessible phenolics, especially flavonoids, which results that are in a high content, with the extraction method previously described. The imbibition stage showed to be an active period of phenolic antioxidant synthesis for our sprouts (Fig.2). These results are in agreement with another work on different seeds germination which is made by Bolívar A. Cevallos-Casals, Luis Cisneros-Zevallos [14]. From the day five until the seventh day, *Medicago sativa* sprouts have not a high content in phenolic acids, but the flavonoids and flavones are significant and this is reflecting in the Figure 3. Nevertheless, the phenolic profile may also vary with seeds quality and environmental conditions under which they grow including temperature, humidity and length of germination [15]. These compounds are involved in plant development during seed germination, being depleted throughout this period [15] and can also serve in plant-microbe recognition and signal transduction [16].

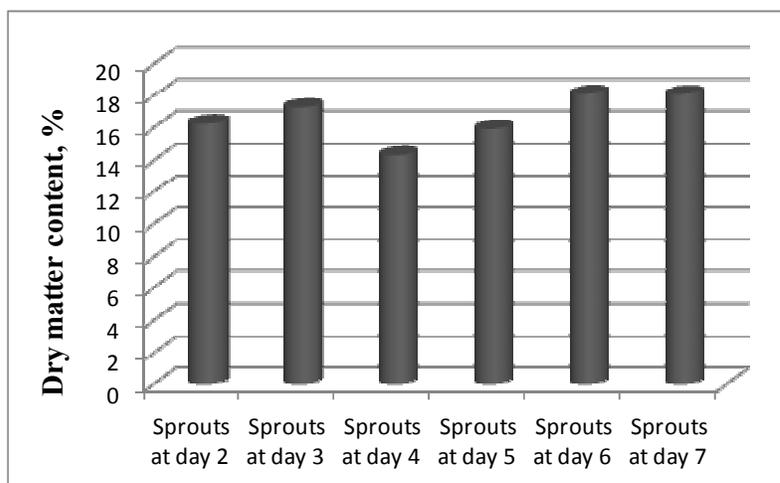


Figure 1. Dry matter content of the alfalfa sprouts from the second until the seventh day of germination

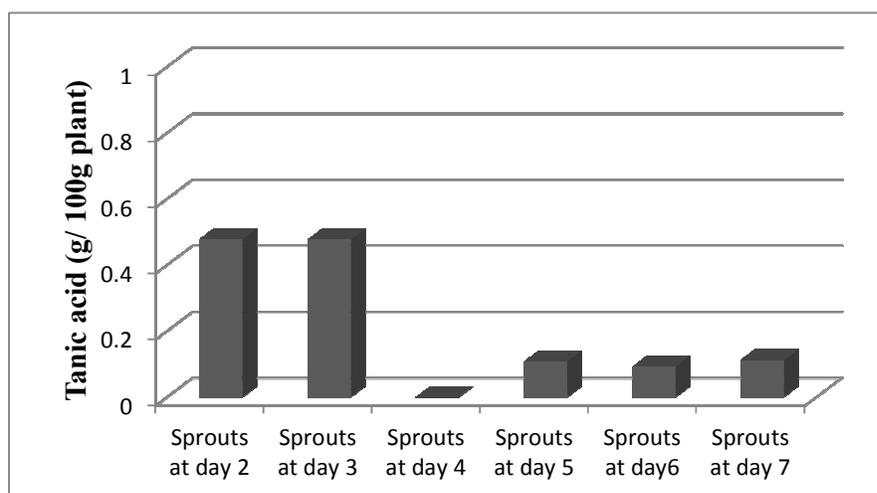
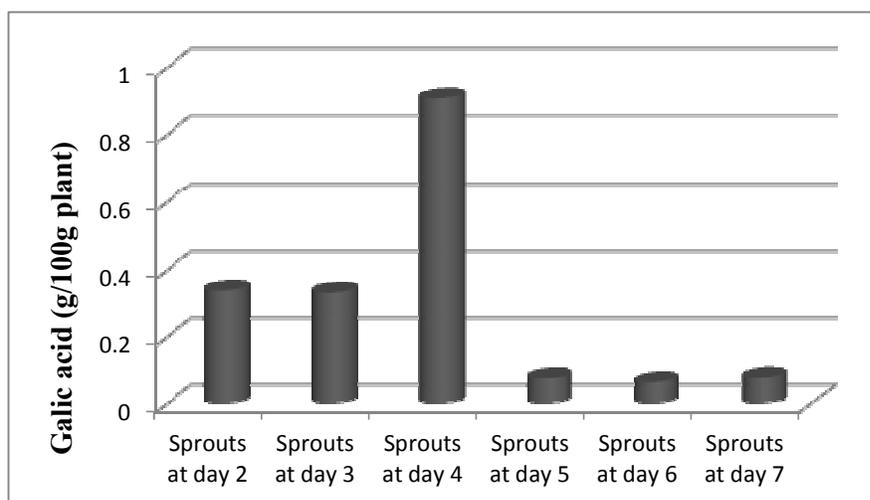


Figure 2. Polyphenolic content of alfalfa sprouts from the second until the seventh day of germination.

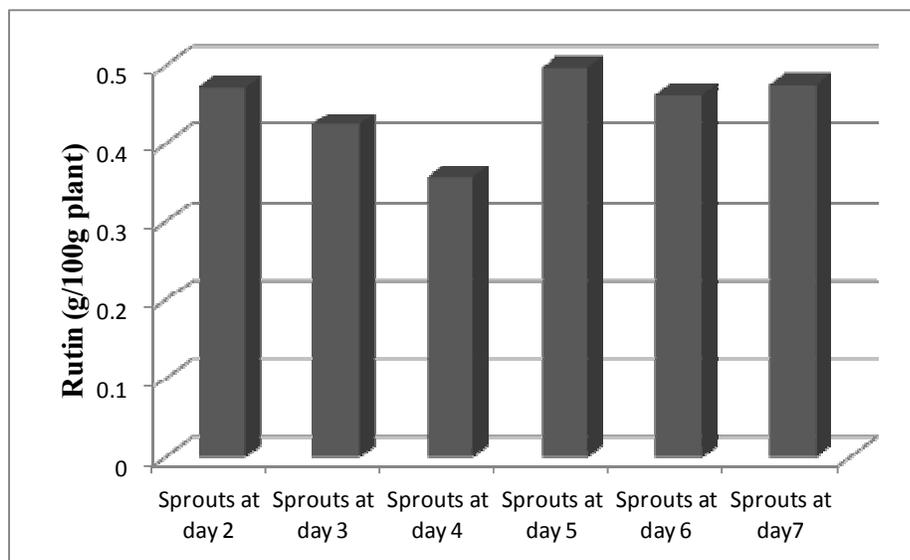
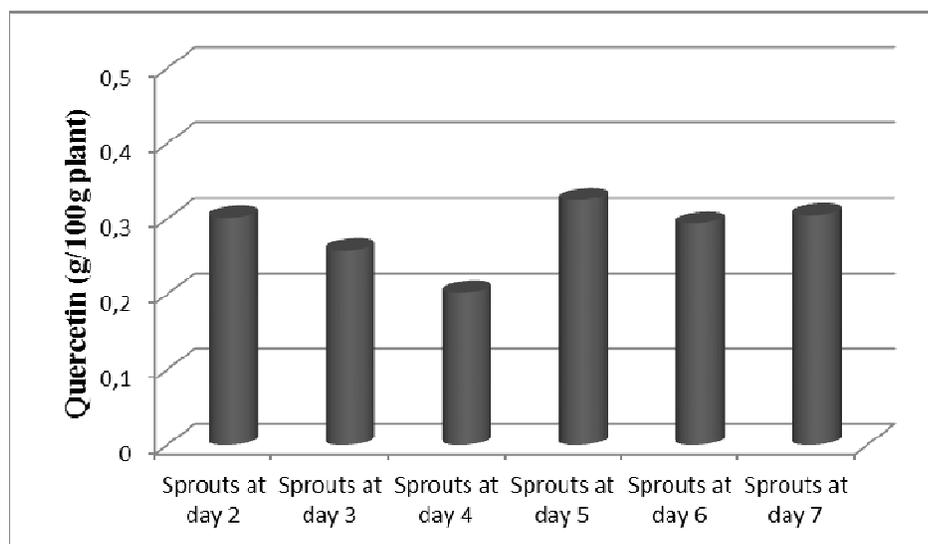


Figure 3. Flavonoids and flavones content of alfalfa sprouts from the second until the seventh day of germination.

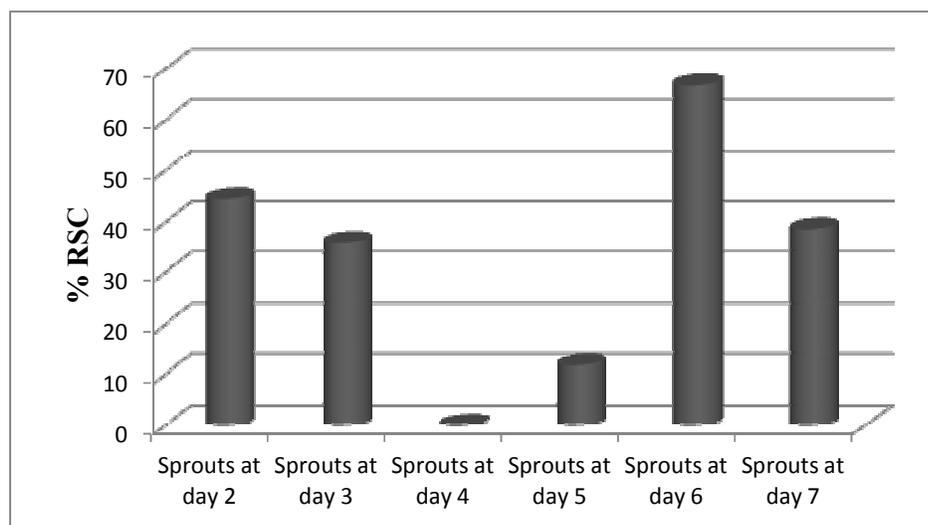


Figure 4. Antioxidant activity of alfalfa sprouts from the second until the seventh day of germination.

The decrease is for the samples from the second, third, sixth and seventh day of germination (Fig.4), this means that in these days of sprouting there has been a high level of antioxidant activity. *Medicago sativa* extract seems to be the most interesting for human health. Different studies established that the antioxidant potential of plant extracts is mainly attributed to the concentration of phenolics [17].

4. Conclusion

Sprouts have the ability to easily develop in a high proportion and in a short time. Have a moisture content which reflecting drying method. This study reflects that at initial germination stages phenolics may serve as radical scavengers or antioxidants. So, the alfalfa sprouts are a very good source of bioactive compounds. Due to the high content of polyphenols, antioxidant activity even reflect this in the second, third, sixth and seventh day of germination. The phenols synthesised during seed germination could help obtain enhanced levels of phenols and antioxidant activity resulting in their improved nutraceutical properties. Seed germination could also serve as models for applying similar nutraceutical enhancement strategies to other crops.

Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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