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Glycine max (L.) Merr., *Vigna radiata* L. and *Medicago sativa* L. sprouts: A natural source of bioactive compounds

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ABSTRACT

The consumption of sprouts, common in Asia, has been growing in western countries, once they are a natural healthy food and considered as a valuable dietary supplement. Comparing with their mature counterparts, sprouts are usually richer in health-promoting phytochemicals. So, the nutritional composition and the biological potential of widely consumed sprouts of three species – *Glycine max* (L.) Merr., *Vigna radiata* L. and *Medicago sativa* L. – were compared for the first time. Phenolic compounds and phytosterols were analyzed by HPLC–DAD and organic acids by HPLC–UV. The volatile profile was determined by HS-SPME/GC–IT/MS. Fourteen phenolic compounds (including four isoflavones), three sterols one triterpene, sixteen fatty acids, seven organic acids and thirty volatile compounds were determined. The antioxidant activity was assessed against DPPH$^{\bullet}$, superoxide and nitric oxide radicals. *G. max* sprouts were the most active against DPPH$^{\bullet}$ (IC_{50} = 1.337 mg/mL), while those of *M. sativa* were the most effective against superoxide and nitric oxide radicals (IC_{50} = 67 μg/mL and IC_{50} = 426 μg/mL, respectively). Data provide evidence of great similarities between *G. max* and *M. sativa* sprouts, both being rich in phenolic compounds, fatty acids and volatiles, and exhibiting better antioxidant activity. On the other hand, *V. radiata* showed higher amounts of sterols, triterpenes and organic acids. In this study it was found that the sprouts are a good source of bioactive compounds in our diet with health-promoting antioxidants.

1. Introduction

In recent years, the consumption of sprouts has been growing worldwide, because they are often perceived as part of a healthy diet (Martínez-Villaluenga, Frías, Gulewicz, Gulewicz, & Vidal-Valverde, 2008). 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 (Randhir, Lin, & Shetty, 2004). Furthermore, the levels of some antinutritional factors, such as trypsin inhibitors, phytic acid and saponins, decrease or even disappear during germination contributing to sprouts nutritional value, while some compounds with antioxidant activity increase, which in turn provide protection against oxidative damage (Doblado, Frias, & Vidal-Valverde, 2007).

Glycine max (L.) Merr., *Vigna radiata* L. and *Medicago sativa* L. sprouts are commonly consumed worldwide. They 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 (Mazur, Duke, Wahala, Rasku, & Adlercreutz, 1998).

Soybean (*G. max*) sprouts contain sterols, tocopherols, minerals, lipids, sugars and phenolic compounds (Kim et al., 2006; Shi, Nam, & Ma, 2010). The consumption of soybean sprouts may reduce the risk of cardiovascular disease and cancer. In addition, studies suggest that these sprouts may also reduce the risk of osteoporosis and help to alleviate menopausal symptoms. Isoflavones are proposed to be the compounds responsible for these beneficial properties because they have both estrogenic and anti-estrogenic effects, depending on the tissue in which they are acting (Prakash, Upadhyay, Singh, & Singh, 2007).

Mungbean (*V. radiata*) is an important pulse crop in many tropical and sub-tropical countries. It is principally grown for its edible seeds and sprouts once they are rich in protein and are good sources of essential fatty acids, tocopherols, sterols, sugars, organic acids, amino acids and amines (Jom, Frank, & Engel, 2010). In addition, their

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sprouts have good digestibility and cause low flatulence (Anwar, Latif, Przybylski, Sultana, & Ashraf, 2007).

Leaves and seeds of alfalfa (*M. sativa*) are also sold as bulk powdered herb, capsules, and tablets for nutritional supplement in health food stores. The sprouts are often consumed as salad and are known for their high content of phenolic compounds, with correspondent high antioxidant activity (Oh & Rajashekar, 2009). The extracts from alfalfa sprouts, leaves, and roots have been indicated to be helpful in lowering cholesterol levels in animal and human studies. In addition, *M. sativa* sprouts or leaves are used in traditional medicine for the treatment of arthritis, kidney problems and boils (Hong, Chao, Chen, & Lin, 2009). This leguminous sprout can decrease H_2O_2-induced DNA damage, which may lower the risk of some cancers (Gill et al., 2004).

It is well known that plant-based diets rich in phytochemicals, primarily with antioxidant properties, can decrease the incidence of chronic and degenerative diseases, including cardiovascular diseases and several types of cancer (Birt, Hendrich, & Wang, 2001).

As far as we know, there are no studies comparing the chemical composition of *G. max*, *V. radiata* and *M. sativa* sprouts, nor their biological activities. Therefore, with this work we aimed to increase the knowledge on the metabolic profile of *G. max*, *V. radiata* and *M. sativa* sprouts and to evaluate some of their biological activities for possible exploitation as food supplements. Following these purposes, phenolic compounds, sterols and triterpenes were determined by HPLC-DAD, organic acids by HPLC-UV and fatty acids and volatile compounds by GC-IT/MS. In addition, the antioxidant and acetylcholinesterase (AChE) inhibitory capacities were checked using microassays.

2. Materials and methods

2.1. Standards and reagents

All chemicals used were of analytical grade. The standard compounds were purchased from various suppliers: citric, quinic, succinic, oxalic, malic, aconitic and fumaric acids, hexanal, octanal, *p*-coumaric and ferulic acids, daidzein, genistein, daidzin, genistin, 3-methyl-1-butanol, 2-methyl-1-butanol, ethyl-octanoate, (*E*,*E*)-2,4-nonadienal, (*E*)-2-nonen-1-ol, 6-methyl-5-hepten-2-one, 2-isobutyl-3-methoxypyrazine, benzaldehyde, linalool, eugenol, (*E*)-2-hexenal, β-cyclocitral, β-sitosterol, campesterol, stigmasterol and betulin were from Sigma-Aldrich (St. Louis, MO, USA); fatty acid methyl esters kit was purchased from Supelco (Bellafonte, PA, USA); methyl jasmonate, 2-methyl-butanal, 3-methyl-butanal, dimethylsulfide, dimethyl trisulfide, (*E*)-2-octenal, 1-octen-3-ol, (*E*)-2-nonenal, (*Z*)-2-heptenal, (*Z*)-2-decenal, 1-penten-3-ol, 3-octen-2-one, (*E*,*E*)-2,4-heptadienal, 2-*sec*-2-methyl-pyrazine, (*E*,*Z*)-2,6-nonadienal, benzyl alcohol, β-ionone, myrtenal and heptanal were acquired to SAFC (Steinhem, Germany); cinnamic, caffeic and 5-*O*-caffeoylquinic acids, eucalyptol, quercetin-3-*O*-glucoside, luteolin-4′-*O*-glucoside, luteolin-7-*O*-glucoside, kaempferol-3-*O*-glucoside and kaempferol-3-*O*-rutinoside were from Extrasynthése (Genay, France) and menthol was from Fluka (Buchs, Switzerland). 1,1-Diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), β-nicotinamide adenine dinucleotide reduced form (NADH), phenazine methosulfate (PMS), nitrotetrazolium blue chloride (NBT), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), sulfanilamide, acetylcholinesterase (AChE) from electric eel (type VI-s, lyophilized powder), acetylthiocholine iodide (ATCI) and sodium nitroprusside dihydrate (SNP), methanol and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-(1-Naphthyl)ethylenediamine dihydrochloride, ethanol, potassium dihydrogen phosphate and sulfuric acid were obtained from Merck (Darmstadt, Germany). Sodium sulfate anhydrous and isooctane were acquired to Panreac Química SA (Barcelona, Spain). Potassium hydroxide was from Pronalab (Lisboa, Portugal) and boron trifluoride (BF_3) 10% methanol solution from Supelco (Bellafonte, PA, USA). Water was deionized using a Milli-Q water purification system (Millipore, Bedford, MA).

2.2. Sprout samples

Sprouts of *G. max*, *V. radiata* and *M. sativa* were purchased from local market and used without any pre-treatment. The samples were frozen, lyophilized and powdered (mean particle size lower than 910 μm).

2.3. Phenolic compounds

2.3.1. Phenolic acids, flavones and flavonols

2.3.1.1. Extraction. Extract's preparation was adapted from Sousa et al. (2007): 3 g of freeze-dried sprouts were boiled for 15 min with 600 mL of water. This procedure mimics the way how the sprouts are usually prepared for human consumption. The resulting extracts were filtered through a Büchner funnel, frozen and lyophilized. The extracts were kept in a desiccator, in the dark, until analysis. For phenolics quantification the extracts were redissolved in ultra-pure water and filtered through a nylon membrane of 0.45 μm (Millipore, Bedford, MA).

2.3.1.2. HPLC-DAD analysis. A published method (Oliveira et al., 2009) was used for phenolic acids, flavones and flavonols determination. Twenty microlitres of each aqueous extract were analyzed on an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0× 0.46 cm; 5 μm, particle size; Waters, Milford, MA) column. The solvent system used was a gradient of water:formic acid (19:1) (A) and methanol (B), starting with 5% methanol and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 50% B at 44 min, 55% B at 47 min, 70% B at 50 min, 75% B at 56 min and 80% B at 60 min, at a solvent flow rate of 0.9 mL/min. Detection was achieved with a Gilson diode array detector. Spectral data from peaks were accumulated in the range 200–400 nm and chromatograms were recorded at 320 and 350 nm, as these correspond to highest absorption of the identified phenolic acids and flavonoids, respectively. The data were processed on Unipoint System software (Gilson Medical Electronics, Villiers-le-Bel, France). Phenolic compounds quantification was achieved by measuring the absorbance recorded in the chromatograms relative to external standards. Phenolic acids were determined at 320 nm and flavonoids at 350 nm. This procedure was performed in triplicate.

2.3.2. Isoflavones

2.3.2.1. Extraction. The procedure described by Shi et al. (2010) was followed. Each powdered sample (0.5 g) was mixed with an acetonitrile/water (5 mL/3 mL) mixture in a vial capped with a Teflon linear cap, under magnetic stirring at 200 rpm for 2 h. Water was then added to make 10 mL. The sample was centrifuged at 4000 rpm during 5 min and the supernatant was filtered by a 0.45 μm polytetrafluoroethylene (PTFE) membrane before HPLC-DAD analysis.

2.3.2.2. HPLC-DAD analysis. Isoflavones were analyzed following a previous procedure (Shi et al., 2010). Twenty microlitres of each extract were analyzed on an analytical HPLC unit (Gilson) coupled to a Gilson diode array detector, using a Spherisorb ODS2 (25.0×0.46 cm; 5 μm particle size) column. The mobile phase consisted of 1% acetic acid in water (v/v) (solvent A) and acetonitrile (solvent B). Elution started with 10% B, and then increased to 20% in 40 min and to 100% in the next 30 min. The flow rate was 0.8 ml/min. Spectral data from peaks were accumulated in the 200–400 nm range. The data were processed on Unipoint System software (Gilson Medical Electronics,