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Germination Effect on Phenolic Composition and Antioxidants Activities of Faba Bean Cultivar

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Abstract

Faba bean (*Vicia faba* L.) is considered as one of the most important legume crops in the world, and fourth on the level of pulses in importance, and it is being grown around the world as a food source of proteins, carbohydrates, fibers, and antioxidants. Germination process have an important effect on the amelioration of the nutritional proprieties of different seeds. The aim of this study was to evaluate the effect of germination on total phenolic content, flavonoids, tannins, total antioxidant capacity and DPPH scavenging activity for faba bean seeds. The characterization of the phenolic compounds was also achieved using HPLC-UV-DAD. The data showed that germination process ameliorate the total phenol and flavonoids contents and DPPH scavenging activity. On the other hand, tannins content and total antioxidant capacity were slightly increase during sprouting process. The result of the HPLC-UV-DAD analysis was also done for the raw and germinated ethanol extracts. One compound, the ascorbic acid (vitamin C) was detected in both samples.

Keywords: **Faba bean, germination, phenol, flavonoids, tannin, antioxidant activity.**

Introduction

Legumes are the second largest plants family, with six subfamilies (Duparquetioideae, Papilionoideae, Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, and Caesalpinioideae) and a lot of species (Azani et al., 2017). Faba bean (*Vicia faba* L.) or broad bean is considered as one of the most important crops in the world, and fourth on the level of pulses in importance, and it is being grown around the world as a food source (Etemadi et al., 2018, Neji et al, 2021, Benmoussa et al, 2022). Legumes are rich in high quality of carbohydrates, fibers and proteins and had an important health benefit (Rajhi et al, 2022a). It contains vitamins, carotenoids and essential minerals containing potassium, magnesium, zinc, iron, selenium, and copper. Faba beans are a considerable source of antioxidants, and they have a lipid lowering effects. Germination process have

an important effect on the amelioration of the antioxidant's properties of different seeds (Ray and Georges 2010, Rajhi et al, 2022b). This antioxidant capacity is acquired from the synergy of a large category of antioxidants ncluding vitamins C and D and polyphenols, mostly flavonoids, phenolic acids, Maillard compounds, trace of minerals, terpenoids and carotenoids (Pérez-Jiménez et al. 2008). It has been demonstrated that natural antioxidant decreased the oxidative stress preventing cells against the negative consequences of the peroxidation of lipid, the denaturation of protein, DNA destruction and reactive oxygen species accumulation which can be responsible for different human disorders such as cancer, atherosclerosis, ischemia–reperfusion damage, neurodegenerative injury, and the aging process (Osawa, 1999; Kranner et al. 2002; Mittler, 2002; Rajhi et al, 2011).

Germination begins when the dry grains begin to absorb water and is finished when the embryonic axis draws out. In this stage, grain reserves within the storage tissues are mobilized to be used by the seedlings during the growth (Benmeziane-Derradji, 2019). From the time of the grain breaking dormancy, protective responses emerge through the synthesis of phenolics and other compounds (Benmeziane-Derradji, 2019). During sprouting time, the level of phenolic antioxidants is increased. Germination process have an important effect on the amelioration of different seeds, so the present experiment aimed to evaluate the effect of sprouting process on the total phenol, flavonoid, and tannin contents and total antioxidants capacity and DPPH scavenging activity in raw and germinated faba bean seeds.

Materiels and Methods

Preparation of Plant Extracts

Flour of vicia faba minor called Saber cultivar was subjected to immersion extraction of phenolic compounds using ethanol (1:10 w/v) and mixed for 24 h at room temperature. The extracts were centrifuged for 15 min at 4,000 rpm and filtered through filter paper (Whatman No 41). The solvents were then removed from the extracts using a rotary evaporator at 40°C under a vacuum (Ksouri et al., 2009). The dried samples were kept in dry clean black glass bottles at 4°C for further analysis. All samples were analyzed in triplicate.

Assay of Total Phenolic Compounds

Total phenolic contents were assessed according to Ksouri et al. (2009). This method is based on the color reaction of Folin-Ciocalteu reagent with hydroxyl groups. The extracts were diluted in proportion of 1:10 with ultra-pure water and then 125 μL of diluted extract was added to 125 μL of Folin-Ciocalteu reagent and 500 μL of distilled water. The mix was agitated before the addition of 1250 μL of Na_2CO3 (7%) for neutralization. After incubation of the samples in dark for 90 min at room temperature, the absorbance at 760 nm of different mixtures versus blanks was measured using a spectrophotometer. The unit of total phenol amounts of different grain parts used is milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Total Flavonoid Content

The measurement of flavonoid contents in plant extracts was well described in Ksouri et al. 2009. A volume of 75 μL of a 5% $NaNO_2$ was supplied to an aliquot of samples or (+)-catechin standard solution. The mixture was shacked for six minutes before adding 150 μL of $AlCl_3$ solution (10%). After the addition of 500 μL of NaOH (1 N), the distilled water was used to adopt a final volume (2. 5ml). The absorbance used for the determination of the flavonoids amounts is 510 nm. The used unit was mg catechin equivalent per gram of dry weight (mg CE/ g DW) through the calibration curve of (+) - catechin, ranging from 0 to 500 μg/mL. All samples were analyzed in triplicate.

Total Tannin Content

The amount of condensed tannin was determined according to Ksouri et al., (2009). Briefly, 50 μL of diluted shoot extracts was mixed with 3 mL of 4% vanillin solution in ethanol and 1.5 mL hydrochloric acid (1 N). The mixture was allowed to stand for 15min, and the absorbance was measured at 500 nm against 80% ethanol. Results were expressed as mg catechin equivalent per gram of dry weight (mg CE/g DW). All samples were analyzed in triplicate.

DPPH Assays

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) electron donating ability of faba bean extracts was determined using a method of Hatano et al.,

(1988). A volume of 250 μL of DPPH methanol solution (2 mmol/L) was supplied to 1ml of extract at different concentrations. Then the mixture was shaken vigorously and incubated for 30 min at room temperature in darkness. The absorbance of the resulting solutions was read at 517 nm. The inhibition concentration corresponded to the ability of extracts to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The extract concentration required to cause a 50% inhibition is expressed as IC50 (μg/mL) and calculated using the following equation: [(A0-A1)/A0]*100 (Hatano et al., 1988). Where, A0 and A1 are the absorbances at 30 min of the control and the sample, respectively. All the analyses were performed in triplicate.

Total Antioxidant Capacity

An aliquot of 100 μL of faba bean extracts was supplied to a volume of 1ml of reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate and 0. 6 M sulfuric acid). The mixture was then incubated at 95°C for 90 min in a thermal block and subsequently the tubes were cooled at room temperature. The absorbance of the samples against the blank was measured at 695 nm. The used unit of total antioxidant activity was mg GAE/g DW (Ksouri et al. 2009). The calibration curve was established between 0 and 500μg/mL. All samples were analyzed in triplicate. All the analyses were performed in triplicate.

Identification Of Phenolic Compounds using HPLC-UV-DAD

HPLC-UV-DAD analysis was performed on LC Agilent Technologies 1100 Infinity series (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto-sampler model 1100, a quaternary pump model 1100, and diode array detector (DAD) model 1100. A C18 column (250 mm × 4.0 mm, 5 µm, Bischoff) was used for analysis. The mobile phase was composed of two solvents: 0.025% trifluoroacetic acid in H_2O and acetonitrile. The sample was prepared at a concentration of 25 mg mL−1 in methanol/H_2O (1:1) and filtered through a 0.45 µm Millipore filter. The elution program at 1 mL min−1 was as follows: 10–50% B (0–40 min), 50–100% B (40–41 min), 100% B (41–50 min), 100–10% B (50–55 min), and 10% B (55–59 min). The injection volume was 25 µL and peaks were monitored at 280 nm. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards. The contents of the identified compounds were obtained from calibration curve with standards.

Results

Effect of Germination on Total Phenol Content

The changes of total phenolic compounds extracted from faba bean seeds before and after germination are shown in Table 1. Results demonstrated that raw faba bean extracts displayed 7.69 mg EAG/g DW. However, a great fold increase was registered for germinated faba bean extracts (raw vs. sprouted 3.61-fold increases). Indeed, the germinated pulse displayed around 27,79 mg EAG/g DW.

Tannin Contents

The quantity of tannins measured in raw and sprouted faba bean cultivar was presented in Table 1. Table 1 showed that germination slightly improves this parameter. Indeed, the tannin contents of raw and sprouted seeds were 4.09 and 5.6 mg EC g DW, respectively.

Flavonoid Contents

In contrast to tannin contents, there is great variation in level of flavonoids among faba bean extracts before and after germination process. Table 1 showed the significant increase of flavonoids produced by germination process. The value of flavonoids detected in raw faba bean ethanol extracts was 1.46 mg EC/g DW. The most surprising effect of germination that the total flavonoid contents of sprouted seeds were increased 22 times in compared to raw seeds (32.38 mg EC/g DW).

Total Antioxidant Activities

The unit used to identify the total antioxidant activity of samples was the number of gallic acid equivalent (mg EAG/g DW). The evaluation of the antioxidant activities in non-germinated and germinated seeds was summarized in Table 1. Faba bean extracts showed a slight augmentation in comparison to raw flour extract (155.11 and 131.88 mg EAG/g DW, respectively).

A free DPPH Radical-Scavenging Activity

The scavenging potential of ethanol faba bean extracts on the DPPH radical was expressed in the terms of inhibitory concentration (IC50). The DPPH stable radical was used for the establishment of the free radical-scavenging activities. The highest antioxidant activities were showing the lowest absorbance. The value of DPPH radical of the raw and sprouted extracts was 275 and 135 µg/ml, respectively. That means that germinated faba bean extract was most significantly less potent against DPPH synthetic radical to that of raw extract. So, germination process ameliorates the scavenging activities of faba bean seeds.

Table 1. Phenolic contents and antioxidant activities of raw and germinatated faba bean seeds

	Raw seeds	**Germinated seeds**
Total phenol content (mg EAG/g DW)	69 ± 1,831	27,79 ± 0,104
Condensed tannins content (mg EC/g DW	09 ± 0,05	5,6 ± 0,076
Total flavonoid content (mg EC/g DW)	47 ± 0,068	32,38 ± 0,032
Total antioxydant activities (mg EAG/gMS)	31,88 ± 0,509	155,11 ± 0,126
Inhibition percentage of DPPH (%)	75 ± 0,586	135 ± 0,658

Data are giving as mean ± sd.

HPLC-UV-DAD analysis

The characterization and separation of phenolic compounds was achieved by HPLC procedures. Figure 1 showed the result of the HPLC-UV-DAD analysis which was done for the 2 ethanol samples; raw and germinated. One compound, the ascorbic acid (vitamin C) was detected in both samples. The retention time of AA (ascorbic acid) was 5.4 min.

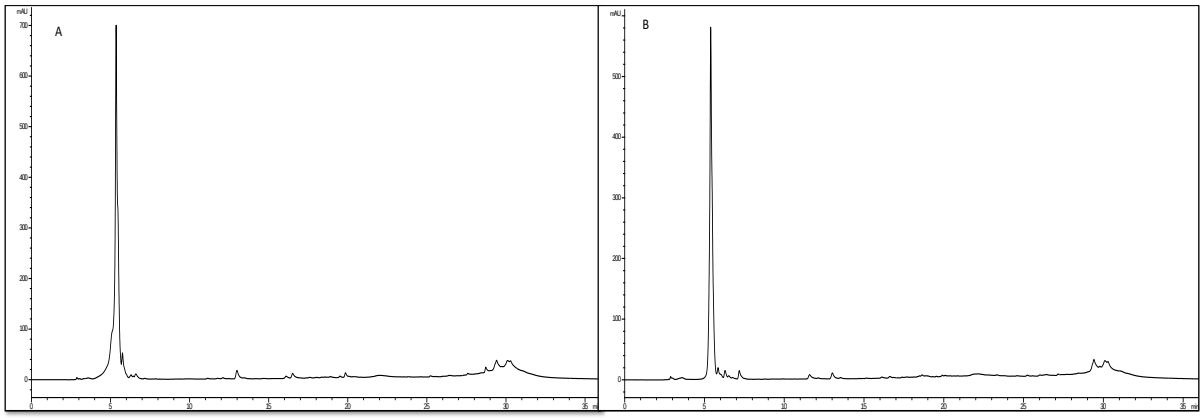

Figure 1. HPLC-UV-DAD chromatograms of ethanol extracts of raw faba bean seeds (A) and germinated seeds (B). (1) Ascorbic acid.

Discussion

The flowering plant Vicia faba, commonly known as the broad bean, fava bean, or faba bean, horse bean, field bean, bell bean, Windsor, or tic bean, is native to North Africa and south-western Asia and is widely cultivated abroad (Rajhi et al., 2020, Neji et al., 2021, Rajhi et al, 2023).

Germination is an economic efficient and inexpensive process that furnishes an important content of bioactive constituents (Duenas et al., 2016; Lopez-Amoros et al., 2013, Rajhi et al, 2022c). Besides, sprouted legumes were considered as a powerful approach to boost antioxidant activities (Swieca et al., 2012, Rajhi et al, 2022d). For long time, sprouted seeds have been largely consumed as food ingredients because they offer significant nutritional benefits compared to unsprouted grains.

Food legumes mainly contain phenolic acids, flavonoids and condensed tannins (Amarowicz, and Pegg, 2008). Germination has shown to have

a significant impact on the phenolic composition and antioxidant properties. The generation of the phenolic acids, condensed tannins, and flavonoids during germination and plant growth and development is a natural process that might help plants to alleviate the harmful effect of environmental conditions (Khang et al., 2016). The augmentation of the phenolic level of germinated pulses is considered as a consequence of the solubilization of condensed tannins when the seeds were imbibed in water and the movement of the phenolic constituents to the outer layer and that can explain the brown color of germinated seeds (Sokrab et al., 2012, Rajhi et al, 2022e). In our study, a significant increase in the total phenolic content was registered in germinated faba bean seeds compared to non-germinated ones. Our results were in accordance with Duenas et al. (2016) and Lopez-Amoros et al. (2006) which indicate that germination ameliorates the quantity and the quality of these compounds of pulses including chickpea, lentil, common vetch, and in dry beans. Thus, the augmentation of seed phenolic values after or during the germination process modifies the functional proprieties of different legume seeds as a consequence of the variation in antioxidant activity. In addition, tannin is implicated in the plant mechanisms defense to fight abiotic stress. Germination displays as well a slight increase in tannin contents in grain legume. Some researchers observed that germination increased the level of tannin in legumes including lupin (Fernandez-Orozco et al., 2008), chickpeas (Khattak et al., 2007) and Beach pea (Chavan et al., 2001). Nevertheless, other researchers reported that germination decreased the content of tannin in different legumes such as kidney beans (Shimelis and Rakshit, 2007), peanuts (Duenas et al., 2009), red beans, and cowpea (James et al., 2020). In our study, germination has a positive effect on the level of flavonoids in faba bean sprouts compared to their raw seeds. Flavonoids have a crucial role in the mechanisms of plant defense. Furthermore, flavonoids are involved in a wide range of biological activities, which has been largely described in the literature (Simons, 2011).

These results occur with previous studies, in which the authors showed the positive effect of germination on oil bean seeds (James et al., 2020). However, the same researchers showed that germination decrease the flavonoid content of cowpea, red bean and pigeonpea.

Germination induces important changes in bioactive compounds of legumes. The antioxidant activities of the raw and germinated faba bean seeds were evaluated by the assay of total antioxidant activities and DPPH methods. The result revealed that germination ameliorates the total antioxidant activities in studied cultivar. It has been reported that the total antioxidant activities in the DPPH assay of cowpea, red bean and groundnut exhibited the highest content after three days of germination (James et al., 2020). In the present work, the 6-day germination effect ameliorates the scavenging activity of faba bean cultivar. The antioxidant activities are closely related to phenolic content (Elzaawely and Tawata, 2012). The greater antioxidant in the DPPH assay recorded by Chourouk cultivar is explained by the highest amount of flavonoids detected in its seeds compared to other cultivars.

Conclusion

In this study, we performed the assessment of the effect of germination on the phenolic compounds and antioxidants activities of faba bean cultivar. The results of this study indicated that germination causes changes in both phenolic and antioxidants activities. Our data showed that the phenolic content and antioxidant activities were ameliorated in germinated seeds. Finally, we can propose sprouted Vicia faba (Saber cultivar) as a source of natural antioxidants which can be used in functional foods or as additives in some medicine formulations.

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