

Original Research Article

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Impact of Malting on Antioxidant Content and DPPH Scavenging Activity of *Medicago sativa*

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ABSTRACT

Medicago sativa is well-known medicinal herb found in India that have high amount of bioactive components which attribute to a strong free radical scavenging activity. In view of the above aspects the present work was undertaken to study the antioxidant potential (total phenols, total flavonoids and ascorbic acid content as well as DPPH scavenging activity) of unmalted and malted seeds with slight modification of standard methods. The study revealed that showed that malted seeds had significantly higher content of total phenols ($41.8 \pm 0.09 \text{ mgGAE/g}$), total flavonoids ($13.5 \pm 0.02 \text{ mgQE/g}$) and ascorbic acid content (8.5 ± 0.05) as compared to unmalted seeds which was significantly increased by 17.7%, 16.3% and 15.9% at $p < 0.05$ level. Whereas, malted seeds exhibits highest antioxidant activity with IC_{50} value ($70.1 \mu\text{g/ml}$) followed by unmalted seeds ($86.7 \mu\text{g/ml}$). Thus, it is concluded that malting as a processing could be used effectively to enhance the antioxidant potential of seeds.

Keywords

Medicago sativa,
Total phenols
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Introduction

Legumes play an important role in the mostly vegetarian diet of the Indian population and are consumed regularly in every household at least in one meal (Gujral *et al.*, 2013). India is the largest consumer and importer of pulses in the world. They contain good amount of carbohydrates, proteins, fibres and bioactive

compounds such as phenols, flavonoids phytosterols, lignans and many other components. These components are associated with lowering the risk of metabolic disorders i.e. type II diabetes, cardiovascular, cancer and many other diseases (Pudnez, *et al.*, 2014). They are also supposed to possess antioxidative, anti-inflammatory, antiallergic and anticarcinogenic activities due to high

concentration of phenolic compounds (Nderitu, *et al.*, 2013).

Medicago sativa commonly known as alfalfa is a perennial species belongs to the *leguminosae* family. It is one of the genus and most reputed medicinal plants which has the largest cultivation area because of its numerous qualities such as cold resistance, salt tolerance, high yield, soil amelioration and economic benefits (Shangli *et al.*, 2017).

It is considered as ‘father of all plants’ and green food of the millennium and one of the remarkable legumes that contain high amount of protein, calcium, vitamins in order to maintain proper functioning of the whole body (Dong *et al.*, 2011). It also contains high amount of pharmacologically active components including acids (lauric, maleic, malic, malonic, myristic, oxalic, palmitic, quinic), saponins, alkaloids, flavonoids, glycosides, phytoestrogen and phenols with various pharmacological properties such as antimicrobial, anti-inflammatory, anticancer, antihypercholesterolemic and antidiabetic (Rathee *et al.*, 2009).

Several methods have been generally adopted to improve the nutritional and organoleptic qualities of legumes. Malting has been suggested as an inexpensive and effective way to improve the quality of legumes and for the elimination of the nutritional impediments of foods. It is a biotechnological technique which involves the controlled germination of a cereal grain (Laxmi *et al.*, 2015) by which the quality of a cereal can be improved for both digestibility and physiological function, particularly through the breakdown of certain anti-nutrients, such as phytate, tannin and protease inhibitors (Graham *et al.*, 2000). In this regard, the main objective of the present research is to study the impact of malting on antioxidant potential of *Medicago sativa*.

Materials and Methods

Collection of seeds

Medicago sativa (alfalfa) were purchased from the local market of Haldwani and authenticated by Agronomic and Horticulture Department of GB Pant University of Uttarakhand. The flour was prepared by malting technique which includes Soaking (3h), Germination (48h) and Kilning (4h).

Preparation of malted flour

500g of seeds were weighed, stored and steeped in distilled water for 3 h at room temperature and rained seeds were spread on a moistened jute sack and allowed to germinate at room temperature (2 days).

The germinated grains were manually washed with distilled water, drained and kilning was done at 60°C for 4 h. The dried grains were milled using mixer grinder, sieved and put into airtight container under room temperature for further analysis.

Preparation of aqueous extract

20g of seeds powder was kept in 200ml conical flask and 100ml distilled water was added.

The mouth of the conical flask was covered with the aluminium foil and kept in a reciprocating shaker for 25 minutes for continuous agitation at 150 rpm for thorough mixing. Then extracts were filtered by using muslin cloth followed by whatman filter paper No. 42 (125mm).

The contained was filtered by using rotator vacuum evaporator with the water bath temperature of 65°C and finally the residues were collected and used for the analysis (Nagappan, 2012).

Determination of antioxidants content

Determination of total phenols content

Total phenols content were determined by Folin-Ciocalteu Reagent using gallic acid as a standard phenolic compound. A dilute extract of tuber (0.5 ml of 1:10g/ml) or Gallic acid was mixed with Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and the mixture was stirred vigorously. 4ml of aqueous sodium carbonate (1M) was added after 3 minutes and then allowed to stand for 2 h with intermittent shaking. After that, the absorbance was measured at 765 nm in spectrophotometer against blank consisting of all the reaction agents except the extract. Total phenols content values are expressed in terms of Gallic acid equivalent (mgGAE/g of dry mass) (McDonald *et al.*, 2001).

Determination of total flavonoids content

Total flavonoids content were determined by using aluminium chloride colorimetric assay. A volume of 125µl of tuber extract is added to 75µl of a 5% NaNO₂ solution. The mixture was allowed to stand for 6 min. 150µl of aluminium trichloride (10%) was added in it and incubated for 5 min, followed by the addition of 750µl of NaOH (1M). The final volume of the solution was adjusted to 2500µl with distilled water. After 15 min of incubation of mixture turned to pink and the absorbance was measured at 510nm using spectrophotometer. The total flavonoids content was expressed as mg of quercetin equivalent (mgQE/g dry mass) (Ebrahimzaded *et al.*, 2008).

DPPH radical scavenging activity

The ability of the aqueous extracts to scavenge free radicals was determined against a very stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) determined

Spectrometric method. Aliquots of the sample extract at different concentrations 20-200 µg/ml were added to 1 mm aqueous solutions of DPPH. Each mixture was vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage. IC₅₀ value was also determined by graph (Nithianantham *et al.*, 2008). DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The results obtained were expressed as Mean±SD and Paired t-Test of three determinations and also statistically analysed to ascertain its significance. The significance was estimated at (p≤0.05level).

Results and Discussion

Table 1 showed that the total phenolic content (mgGAE/100g) and total flavonoids content (mgQE/100g) of unmalted seeds aqueous extract were 35.5±0.02 and 11.6±0.17 respectively. Whereas, malted seeds extract had 11.6±0.17 and 13.5±0.02 total phenol and flavonoids content which were significantly increased by 17.7% and 16.3% at p≥0.05 level when compared with unmalted seeds extract. Similar study was conducted by Aguilera *et al.*, (2013) who reported that germinated seeds of *Stizolobium niveum* contained significantly higher total phenols content (46.30±0.12mgGAE/100g) when compared with ungerminated seeds (37.40±0.42mg GAE/100g) and significantly increased by 23.7% which is agreement with the obtained results. The value obtained compared favourably with Carciochi *et al.*, (2016) who reported that malted seeds of quinoa had significantly higher total flavonoids content

i.e. 17.65 mgQE/100g when compared with unmalted seeds(11.06 mg QE/100g) and significantly increased by 59.5% at $p \geq 0.05$ level. The ascorbic acid content (mg/100g) of unmalted and malted seeds extract was 3.81 ± 0.16 and 8.5 ± 0.05 respectively. This shows that ascorbic acid content of malted seeds was significantly increased by 15.9% at

$p \geq 0.05$ level when compared to unmalted seeds. The present study is comparable with Laxmi *et al.*, 2015 who reported that malted chickpeas had significantly higher vitamin content i.e. 7.1mg/100g as compared to unmalted seeds (3mg/100g) which was significantly increased by 136.6% at $p \geq 0.05$ level.

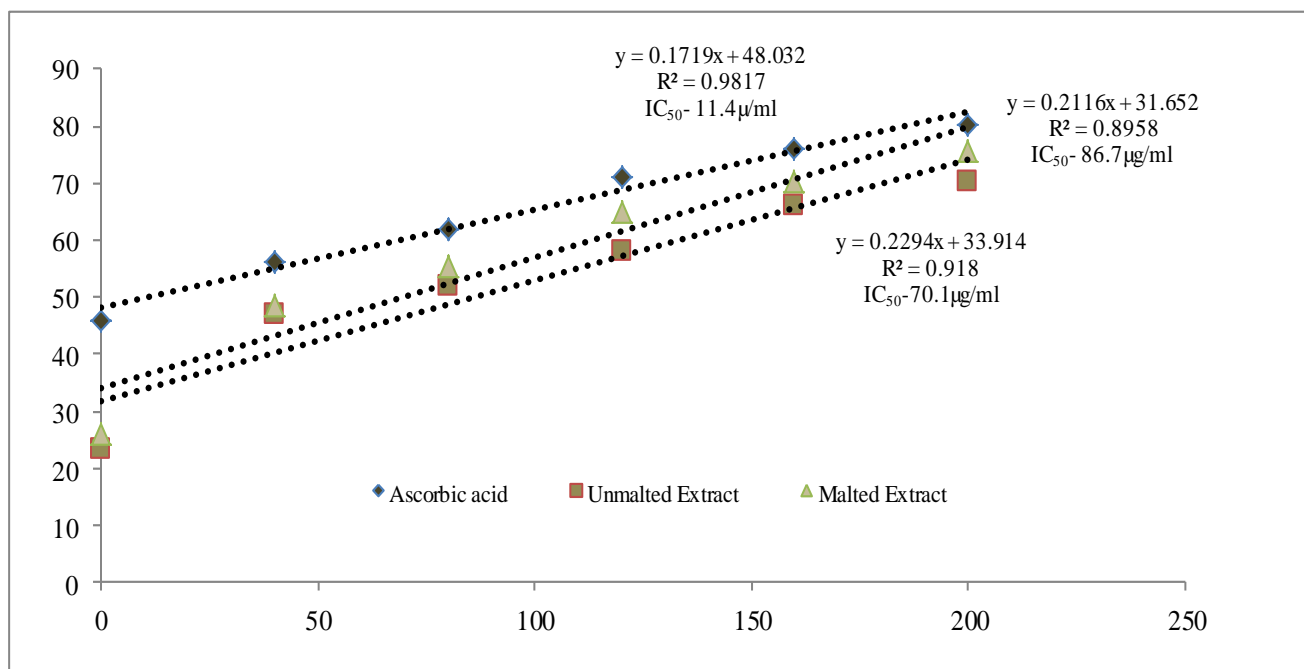
Table.1 Antioxidant Content of Aqueous Extract of Unmalted and Malted *Medicago sativa*

Antioxidants	<i>Medicago sativa</i> Extract	
	Unmalted	Malted
Total Phenols Content (mgGAE/100g)	35.5±0.02	41.8±0.09*(17.7%↑)
Total Flavonoids Content (mgQE/100g)	11.6±0.17	13.5±0.02*(16.3%↑)
Ascorbic Acid (mg/100g)	3.81±0.16	8.5±0.05*(15.9%↑)

Values are expressed as Mean ±SD of triplicate determinations of unmalted and malted seeds flour.

*Shows significant difference at ($p \geq 0.05$) level at ($p \geq 0.05$) level

Figure.1 % DPPH Radical Scavenging Activity of Aqueous Extract of Unmalted and Malted *Medicago sativa*



In vitro activities of both unmalted and malted seeds extracts were measured with the standard antioxidant (Ascorbic acid). Here, the DPPH free radical scavenging activities of unmalted and malted alfalfa seeds is shown in Figure 1. The malted seeds exhibits highest antioxidant activity with IC₅₀ value (70.1µg/ml) followed by unmalted seeds (86.7µg/ml) as compared to control (11.4µg/ml). Thus, aqueous extract of malted seeds showed strongest antioxidant activity as compared to unmalted alfalfa seeds extract. Similar study demonstrated by Gomathi *et al.*, 2016 who reported that DPPH scavenging activities of raw and germinated alfalfa seeds extract with IC₅₀ values were 61.9µg/ml and 80.02µg/ml respectively. Whereas, Simic *et al.*, 2017 have revealed that DPPH scavenging activities of unmalted and malted barley grain extract with IC₅₀ values were 62.2µg/ml and 68.1µg/ml.

In conclusion, the study indicates that malting had significantly affected the total phenols, total flavonoids and ascorbic acid content as well as antioxidant activity of seeds. Thus, it might be concluded that malting as a processing can be used effectively to enhance the antioxidant potential of seeds.

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