Antioxidant activity and total phenolic contents of dried and germinated legumes

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Abstract

Beans and lentils are good sources of total phenolic contents and possessed excellent free radical scavenging activity. This study was aimed to assess the total phenolic compounds (TPCs) and antioxidant activity of dried and germinated beans and lentils. The total phenolics contents were extracted from seven types of beans and lentils by using 50% (v/v) aqueous ethanol. The TPCs and antioxidant activities were analyzed using Folin-Ciocalteu and DPPH (2, 2-diphenyl-1-picrylhydrazyl radical), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) assays, respectively. The legumes showed the highest total phenolic contents (TPCs) and antioxidant capacity by DPPH and ABTS scavenging activity ($p < 0.05$) in germinated samples. The germinated beans and lentils contained more total phenolic contents that decrease significantly in dried beans. This study also suggested that germinated beans are a valuable source of commercial natural antioxidants.

Introduction

Legumes are cultivated and an essential part of human meals throughout the world¹. The consumption of phenolic rich legumes is maximum in Asia than other continents due to therapeutic effects of numerous bioactive compounds that influence the human metabolic machinery². The legumes also contain protein and minerals with low glycemic index) and also maintain the bone health³.

Legumes contain many antioxidant compounds like anthocyanins, procyanidins, tannins, flavonoids (flavanols) and phenolic (Flavan-3-ols) derivatives⁴, which combat with cardiovascular diseases⁵, breast cancer and provide protection against hypercholesterolemic effects.

The polyphenols of legumes play a vital role in metabolic and physiological processes⁶, which hinder the production of free radicals formed by the breakage of biological molecules (lipids, protein, DNA) in our body⁷. The water insoluble polyphenols act as anti-atherogenic, anti-inflammatory, antimutagenic and antimicrobial activities⁸.

Germination stage of legumes causes change in biological activities due to these compounds during respiration and new cell formation. This is an efficient stage to enhance the nutrient activities (amino acid, dietary fibers, and soluble sugar) and various other compounds of legume and pulses⁹.

The aim of this study was to explore the total phenolic compounds (TPCs) and antioxidant activity of dried and germinated beans and lentils.

Materials And Methods

Sample preparation

Five types of beans including white chickpeas (*Cicer arietinum*), black chickpeas (*Cicer arietinum*), red beans (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), mung beans (*Vigna radiata*) and two types of lentils masoor (*Lens culinaris*) and white lentils (*Vigna mungo*) were selected for analysis and purchased randomly from Gulberg, Lahore, Pakistan in 2019. Each sample was divided into six equal portions. Three portions of each sample were analysed for antioxidant activity of total phenolic contents, while the other three were subjected to germination.

Germination of samples and measurement

An aliquot of 2-3 g of each bean/lentil sample was cleaned with running water, and then soaked in water for 1 h at 28°C. After removing excess water, seeds were placed into a sprouter (60 × 150 mm) and kept in the dark at 28°C for germination. The sprouts germination with radicle period was 120 h. The seeds were moistened with water after every 24

h. Incubate at 28 °C till 90% beans were germinated. Elongation of the germinated bean/lentils radicles were taken by measuring scale (cm). Percentage Elongation of radicles were determined by $\%EL = L_f - L_o / L_o \times 100$.

Extraction of samples

Each sample (2-3 g) was grinded and extracted in a capped centrifuge tube with 50 mL solvent of ethanol/water (50% v/v). At temperature (25°C), the mixture was well shaken (300 rpm) for 3 h. After centrifugation, the mixture was placed under darkness for 12-24 h. Supernatant was taken from extracts into a new tube after centrifugation at 4000 rpm for 10 min. Residues left behind in the tube were also extracted with 10 mL of respective solvent. Both extracts were combined and stored at 4 °C in the dark until further analysis.

Preparation of analysis solutions

(A) *Gallic acid standard solution*. Gallic acid stock solution was prepared by mixing 0.100 g gallic acid in 100 mL distilled water. Series of working standards of gallic acid were prepared by taking 10, 20, 50, 100, 150 and 200 µl of stock solution for total 5 mL constituents to prepare a standard curve in the linearity range of 2 µg–40 µg mL⁻¹. (B) DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) solution (0.1 mM). To prepare 0.1 mM of DPPH solution, 39.4 mg of DPPH was accurately weighed and dissolved in 1 L of ethanol. (C) ABTS (2,2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulphonate) solution (0.1 mM). To prepare 0.1 mM of DPPH solution 0.22 mg of ABTS was accurately weighed and dissolved in 1 L of ethanol.

Determination of TPCs

Folin-Ciocalteu reagent was used to determine the total phenolic contents accordingly Xu and Chang¹⁰. Extract (50 µl) was homogenized with 250 µl of Folin-Ciocalteu reagent, 750 µl of 7% Na₂CO₃ (W/V) and 3 mL of distilled water and left for 8 min. Add 950 µl of distilled water again and allow to settle for 2 h in room T. Absorbance (765 nm) was measured by using distilled water as a blank in UV/Visible spectrophotometer (Schimadzu UV 1700). All determinations were done in triplicate (n = 3). The quantity of total phenolic contents was determined from the standard curve of gallic acid and stated as gallic acid equivalent (mg GAE/g) of beans and lentils. The linearity range of the calibration curve of Gallic acid was 2 µg-40 µg mL⁻¹ and $r^2 = 0.9991$ (Figure 1).

Determination of antioxidant capacity (AC)

(A) *DPPH (2, 2-diphenyl-1-picrylhydrazyl radical)*. The method modified by Xu and Chang¹⁰ was used to determine antioxidant activity. Ethanolic DPPH solution of 3.8 mL (0.1 mM) was mixed in 0.2 mL of legume extract. Vortexing this mixture for 1 min and then placed in darkness (30 min). Absorbance was measured at 517 nm of the resulting solution (A_{sample}) by a spectrophotometer (Schimadzu UV 1700) using ethanol as a blank. For the control set, respective extraction (0.2 mL) was added into 3.8 mL of DPPH solution and absorption (A_{control}) was measured at 517 nm. The DPPH discoloration of the sample was calculated in percent according to the equation = $[1 - (A_{\text{sample}} / A_{\text{control}})] \times 100$. The antioxidant content was determined using BHA as an external standard. A calibration curve of BHA was used to calculate the AOs with a unit of micrograms of BHA equivalents per 100 g of dried beans or germinated bean with sprouts (µg BHA/100g) under the same experimental conditions. (B) *ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate)*. Add 3.0 mL of diluted ABTS (0.1 mM) solution (A734 nm) to 0.2 mL of the legumes extracts, the absorbance was taken exactly 10 min after the initial mixing. The ABTS scavenging activity of each seed extract was calculated as a difference between the initial absorbance and that after reacting for 10 min. Methanol was used as the blank solution. All determinations were performed in triplicate. The antioxidant content was determined using BHA as an external standard. A calibration curve of BHA was used to calculate the AOs with a unit of micro grams of BHA equivalents per 100 g of dried beans or germinated bean with sprouts (µg BHA/100g) under the same experimental conditions.

Statistical analysis

Results were expressed as mean $\pm$ standard deviation. Data were statistically analysed using software *SPSS*. Significance difference in total phenolic contents and antioxidant activities between germinated and non-germinated beans and lentils was determined at $p < 0.05$ by applying ANOVA of means. The $p < 0.05$ was considered statistically significant.

Results

Effects of germination time on radicles elongation

Elongation of the germinated bean/lentils radicles exhibited a quick tendency during the germination period of 120 h. The elongation of radicles during germination time of 120 h in cowpea, red beans and lentils masoor was 23.3, 21.5 and 14.5 cm than other beans/lentils. The elongation percentage of radicles during germination time of 72 h was decreased in *Phaseolus vulgaris*, *Lens culinaris*, and *Vigna unguiculata* than other beans and lentils. It suggested that the elongation efficacy of radicles were decreased with the extension of germination time. Hence, the most effective elongation period of radicles was the early 1–3 days of germination (Figure 2).

The percentage (%) elongation of radicles in the germinated beans/lentils were 87.32%, 77.33%, and 72.59% in *Lens culinaris*, *Phaseolus vulgaris*, and *Vigna radiata* sprouts, respectively in a time-dependent manner and reached the peak on 72 h, but the values reduced in percentage elongation from 87.32–56% and 25% on day 4 and 5 respectively in *Lens culinaris*. The elongation percentage of radicles was 48–77.33% and 36% in *Phaseolus vulgaris* and 41–72.59% and 28% in *Vigna radiata* on day 4 and 5, respectively. The least percentage was observed in *Vigna mungo* (25.86%) on day 5 (Figure 3).

Quantity of total phenolic in dried and germinated seeds

The result of our study indicated that TPCs in all beans and lentils have been significantly increased ($p < 0.05$) after germination (Table 1).

Table 1
Comparison in Percentage Increase in Total Phenolic Contents of Dried and Germinated Legumes

Total phenolic contents (mg of GAE/g)			
Common name	scientific name	Germinated beans/lentils	Dried beans/lentils
Red beans	<i>Phaseolus vulgaris</i>	15.4 $\pm$ 0.01a	3.49 $\pm$ 0.41b
Masoor (lentils)	<i>Lens culinaris</i>	11.8 $\pm$ 0.16b	1.49 $\pm$ 0.03d
Mung	<i>Vigna radiata</i>	10.2 $\pm$ 0.08c	2.79 $\pm$ 0.33c
Mash (White lentils)	<i>Vigna mungo</i>	8.93 $\pm$ 0.05b	6.62 $\pm$ 0.55a
Cowpea	<i>Vigna unguiculata</i>	3.5 $\pm$ 0.14d	2.34 $\pm$ 0.07c
Black chick peas	<i>Cicer arietinum</i>	2.8 $\pm$ 0.06e	1.70 $\pm$ 0.23d
White chick peas	<i>Cicer arietinum</i>	2.5 $\pm$ 0.01d	1.83 $\pm$ 0.55c

Literature cited that legume fractions and germination time were affected ($p < 0.05$) on TPC. Total phenolic contents were significantly ($p < 0.05$) increased in a time-dependent manner after the germination than the original concentration of leguminous seeds. In this study, the germinated sample of red beans (*Phaseolus vulgaris*) exhibited the maximum amount of TPCs (15.40 mg GAE/g), which was low in white chickpeas (*Cicer arietinum*) (2.52 mg of GAE/g).

In white lentils of this work, TPCs value was 8.93 mg GAE/g) and 6.62 mg GAE/g in germinated and dried seeds, respectively. The TPCs of *Lens culinaris* (1.49 mg GAE/g) were lower amongst the remaining samples of this study (Table

1).

In this research work, the TPCs of germinated legumes were observed in the following order: Red beans (*Phaseolus vulgaris*) 15.40 > Masoor (*Lensculinaris*) 11.76 > Mung (*Vigna radiata*) 10.2 > Mash (*Vigna mungo*) 8.93 > Cowpea (*Vigna unguiculata*) 3.46 > Black chickpae (*Cicer arietinum*) 2.79 > White chickpeas (*Cicer arietinum*) 2.52 mg GAE/g. The decreasing order of dried legumes were following: Mash (*Vigna mungo*) 6.62 > Red beans (*Phaseolus vulgaris*) 3.49 > Mung (*Vignaradiata*) 2.79 > Cowpea (*Vigna unguiculata*) 2.34 > White chickpea (*Cicer arietinum*) 1.83 > Black chickpeas (*Cicer arietinum*) 1.70 > Masoor (*Lensculinaris*) 1.49 mg GAE/g (Table 1).

The low-molecular-weight antioxidants like phenolic compounds particularly provide the antioxidant capacity to the lentils. Germination time and legume fractions were also influenced on antioxidant capacity. The extracts of germinated and dried legumes exhibited different antioxidant capacity, and the values ranged from 0.74 to 14.01 µg equivalent of BHA/100g and 4.63 to 14.06 µg equivalent of BHA/100g respectively in the case of DPPH with statistically significant differences. The germinated red beans extract has the highest total antioxidant capacity in DPPH (18.59 µg/100g) than dried beans (14.06 µg/100g). Similarly, dried white beans (Cowpea) were lowest (0.74 µg/100 g) than germinated seeds (3.76 µg/100 g). Germinated beans/lentils exhibited better radicle scavenging activity with ABTS rather than DPPH in Mash (White lentils), Cowpea, Black chickpeas and White chickpeas of this study. The ABTS is also better than DPPH in determination of phenolic contents in dried seeds and lentils as well. In this study, germinated lentils (Masoor) contained maximum ACs (14.01 µg/100g) than dried lentils (Masoor) (Table 2)

Table 2
Antioxidant Activity and DPPH•Radical Scavenging Activity of Dried and Germinated Legumes.

Common name	Scientific name	% inhibition of 0.1 mM	% inhibition of 0.1 mM	Antioxidant activity (µg equivalent of BHA/100g)			
		ABTS (GB/L)	DPPH (GB/L)	GB/L(DPPH)	DB/L(DPPH)	GB/L(ABTS)	DB/L(ABTS)
Red beans	<i>Phaseolus vulgaris</i>	82.78±0.07	67.49±3.43	18.59±0.01a	14.06±2.87a	15.01±0.87b	12.06±1.7a
Masoor (lentils)	<i>Lens culinaris</i>	67.31±0.02	4.70±0.30	14.01±0.02b	4.52±0.81c	13.06±2.7c	8.06±1.87c
Mung	<i>Vigna radiata</i>	57.86±0.10	16.20±0.55	11.21±0.01c	1.12±0.94e	11.09±2.17d	10.26±1.2b
Mash (White lentils)	<i>Vigna mungo</i>	50.97±0.08	35.63±1.62	9.17±0.01d	4.63±1.97b	14.6±2.5b	11.6±3.7a
Cowpea	<i>Vigna unguiculata</i>	22.50±0.06	7.28± 2.42	3.76±0.02c	0.74±3.05d	13.87±1.07c	09.8±1.8b
Black chick peas	<i>Cicer arietinum</i>	15.18±0.05	5.90±1.53	1.42±0.04d	4.17±2.05c	21.5±2.2a	8.06±3.0c
White chick peas	<i>Cicer arietinum</i>	12.13±0.06	3.27±0.53	2.32±0.01e	4.95±0.96c	14.06±1.7c	10.02±2.7a
Dried beans/lentils (DB/L), Germinated beans/lentils (GB/L).							

Estimation of radical scavenging activity. DPPH[•] and ABTS^{•+} are a stable, simple and quick organic radical used to estimate the antioxidant capacity useful to evaluate the free radical scavenger activity in legumes. The antioxidant action

of beans is interlinked with the quantity of polyphenols. The antioxidant capacities significantly differ amongst the samples of this work. The observed DPPH and ABTS scavenging capacity of the ethanol-based extracts of beans and lentils were given, which significantly higher in germinated red beans (Table 2). The scavenging activity in percentage inhibition (ABTS) of germinated beans and lentils extract decreased in the following order: *Phaseolus vulgaris* > *Lens culinaris* > *Vigna radiata* > *Vigna mungo* > *Vigna unguiculata* > *Cicer arietinum* (black) > *Cicer arietinum* (white). Whereas in DPPH the order of inhibition in germinated beans/lentils was *Phaseolus vulgaris* > *Vigna mungo* > *Vigna radiata* > *Vigna unguiculata* > *Cicer arietinum* (black) > *Lens culinaris* > *Cicer arietinum* (white) (Table 2).

Significant positive correlation was observed between phenolics and antioxidant activity by DPPH and ABTS scavenging activity in the various legume extracts. The data revealed that germinated beans possessed highest total phenolic contents with antioxidant activity than lentils. The free radical scavenging activity of beans and lentils increased in both germinated beans and lentils along total phenolic contents. It means total phenolic contents are the major contributor in analysis of antioxidant capacity of beans and lentils. In this study, red beans (*Phaseolus vulgaris*) were stronger free radical scavengers, because of high level of total phenolic contents and antioxidant capacity.

Conclusion

The cultivars of beans and lentils processed different amount of total phenolic contents as well as antioxidant activity. The germinated beans and lentils contained more phenolic contents that decrease significantly in dried beans. The germinated beans were valuable source of natural antioxidants and sprouting was the best stage to increase few nutritional ingredients and antioxidants in beans and lentils.

Discussion

Elongation efficacy of radicles was effective in first three days¹¹, similarly to results of this study, contrary to 3 to 4-day-germination in work of Zhou and Zhang¹². Legumes are famous for total phenolic compounds rather than rice, corn, millet and wheat. Major classes of phenolic contents in beans and lentils have been gaining popularity due to antioxidant activity. The levels of natural endogenous antioxidants (e.g., phenolics, tocopherols; vitamin C) vary during seed germination of legumes. Federica et al.⁶ analysed 14 polyphenolic compounds ranging from 3 mg kg⁻¹ for dehulled red lentils to 1630.5 mg kg⁻¹ for ruviotto beans.

Legume fractions and germination time were affected on TPC. The total phenolic contents were increased after the germination. Similarly, the TPC contents were significantly ($p < 0.05$) increased with the germination time in seed hulls, radicles, cotyledons of legume¹³ and lentil seeds. Contrary results were shown by lentils and beans of Aguilera et al.¹⁴ and Duenas et al.¹⁵. No change in TPCs was shown by kidney beans, but the values were highly decreased in lentils¹⁶. Our results regarding TPCs were significantly higher than common bean seed coats (0.69-3.32 mg GAE/g) reported by Chávez-Mendoza et al.¹³.

The high amount of polyphenols were also found in black, brown, red beans¹⁷, adzuki bean¹⁸, and faba bean (40.7 to 66.1 mg g⁻¹)¹⁹ extract, whereas low in pea²⁰, broad beans²¹, lupins²², white beans²³ and grass pea²⁴. The dried beans of our black (1.70 mg GAE/g) and white chickpeas (1.83 mg GAE/g) possessed the lowest value of TPCs compared to Fenugreek (5.79 mg gallic acid/g), (56.14 mg gallic acid/g) and chickpea seeds (5.68 mg gallic acid/g), (57.94 mg gallic acid/g)²⁵.

The TPCs of *Lens culinaris* (1.49 mg GAE/g) were lower amongst the remaining samples of this study (Table 1), similarly in lentils of Salem et al.²⁶ (60.39 mg GAE/g), Nair et al.²⁷ (1191 mg.kg⁻¹) and Awada et al.²⁸ (4730 mg. kg⁻¹), whereas Amarowicz et al.⁷ and Zhao et al.²⁹ demonstrated the highest phenolic content (47.6 mgg⁻¹) in lentils than beans. Contrarily, maximum values were reported in red and green lentils^{7,30}.

There are two schools of thought behind low level of TPCs during germination: 1) sprouting causes the reduction in flavan-3-ols and anthocyanin¹⁵; 2) reactive oxygen species (ROS) released from metabolically active cells of seed, which influenced the biological process of seed germination. ROS as a messenger transmits environmental signals during seed germination and responsible for lowering the antioxidant activity of bean and lentils after germination³¹. TPCs were decreased in peanuts, soybeans and lentils after germination³² contrary to this study (Table 1), while Khang et al.³³ values were significantly increased during germination in all legumes. Zhaohui et al.³⁴ investigated TPCs and antioxidant activity of germinated Mung beans, soybeans and black beans sprouts were highest initially (44.87–90.31%) then decreased.

In white lentils of this work, the TPCs were comparable to faba beans (7.11 mg gallic acid/g)³⁵ and lupine seeds (8.56 mg gallic acid/g)³⁶. Emily et al.³⁷ worked on 14 Canadian pulses included beans, peas, lentils and analyzed antioxidant activity (1.16 to 7.45 mg GAE/g DW) and revealed that samples with dark testa (black lentils and diavoli beans) possessed higher antioxidant activity than pale testa.

Antioxidant capacity (AC) of legumes is due to active micro and macro elements (polysaccharides, vitamins, amino acids proteins)³⁸. In this study, germinated lentils (Masoor) contained maximum ACs than dried lentils (Masoor) similarly the work of Dalaram et al.³⁹ but contrary to lentils of Gubanenکو et al.⁴⁰ and Zhao et al.²⁹. Researchers supported that AOA (Antioxidant activity) increased as the germination time increased²⁵, e.g., in mung beans, soybeans, black beans³³, lentil seeds⁴¹, kidney beans^{14,16}, chickpea seeds, fenugreek seeds, lentil seeds²⁶ and lupine seeds³⁶. Though, black beans did not change AOA during the germination process⁴².

Gubanenکو et al.⁴⁰ revealed that seedlings of lentils show a slightly higher antioxidant activity than chickpea sprouts, whereas seeds of green pea, chickpea⁴³ and faba beans³⁹ dramatically contained maximum TAC. The seed coat of common bean exhibited greater antioxidant capacity (23.86–84.10%) than the cotyledon (0.66–29.77%) of all bean varieties¹³.

The most famous legumes with maximum antioxidant compounds in dietary fibres were found in chickpeas (*Cicer arietinum*), pulses⁴⁴, white and red beans (*Phaseolus vulgaris*), which promote the health and prevent chronic diseases⁴⁵. The result of this study regarding scavenging activity was similar to Saleh et al.²⁵. They explained common beans (84.52%) contained highest radical scavenging activity than lupine seeds (78.29%). The values of lentils scavenging capacity of this study were significantly higher than 38.5%²⁹.

The antioxidant activity of beans increased after germination and decreased in lentils depends upon type and conditions of germination⁴⁶, similarly to this study, where free radical scavenging activity increased in germinated seeds depend on the time taken for germination. Wang et al.⁴⁷ worked on Chinese beans, spring bay beans, black beans, pearl beans and determined the strong positive relationship between total phenolic contents and antioxidant activities. Black, red, green beans, red kidney beans and soybeans possessed higher total phenolic contents and antioxidant capacity, whereas red and yellow lentils (dhal) and chickpea possessed lower capacity⁴⁸.

Conclusion

This study concluded that germinate legumes exhibited the highest total phenolic contents (TPCs) and antioxidant capacity which decreased significantly in dried samples. This study also suggested that germinated beans are a valuable source of commercial natural antioxidants.

Declarations

Conflict of interest

Authors declare no conflict of interest.

Authors' contribution statement

All the authors mentioned in this study contributed equally in this work.

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Authors Contributions

Aisha Umar contributed to complete this work in all aspects.

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Ethics approval

I want to opt traditional publishing model for publishing my this work.

Consent for publication

I allow the journal to publish my work.

Code availability

N/A

Declaration of deposition in repositories

N/A

Experiments on live vertebrates and/or higher invertebrates/human subjects

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Permission to use Plant material

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Data availability" / "Availability of Data and Materials"

N/A

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REASON WHY DATA ARE NOT PUBLIC

N/A

Third party name

N/A

ONLY TO BE INCLUDED IN EMAIL FOR NON-RESEARCH ARTICLES E.G. STUDY PROTOCOLS OR LITERATURE REVIEWS

Not applicable.

Share your raw data

N/A

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Figures

Figure 1

Calibration curve for gallic acid

Figure 2

Kinetic changes of radicle length (cm) of beans/lentils with germination time.

Figure 3

Percentage elongation of radicles (beans/lentils) germination with time.