Int.J.Curr.Microbiol.App.Sci (2018) *7*(5): 3388-3398

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 05 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.705.396

Comparative Evaluation on Phenolic Content and Antioxidant Activity of Legume Sprouts as Affected by Various Solvents for Application in Livestock Products

Serlene Tomar[1], Geeta Chauhan[1*], Annada Das[2] and Med Ram Verma[3]

[1]Division of Livestock Products Technology, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P.-243122, India
[2]Department of Livestock Products Technology, Faculty of Veterinary Science, WBUAFS, Kolkata-700037, India
[3]Division of Livestock Economics and Information Technology, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P.-243122, India

**Corresponding author*

Keywords

Comparative evaluation, Phenolic content, Antioxidant activity, Legume sprouts

Article Info

Accepted:
22 April 2018
Available Online:
10 May 2018

A B S T R A C T

Oxidation process is one of the chief methods for producing free radicals in food, drugs and even living systems. Legumes are a source of not only essential nutrients required for life but also has a number of bioactive compounds required for the sustenance of good health and prevention of diseases. Germination is well known to significantly improve the bioavailability of nutrients and increase the phytochemical content of legumes. In the present study four sprouted legumes (T_1- mung gram, T_2-black gram, T_3-pigeon pea and T_4- Cowpea) were extracted in three different solvent systems (HWE, 60% EHWE and 60%MHWE) and investigated for their total phenolic content (TPC) and *in-vitro* antioxidant activity using different 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging, ABTS assay and reducing power assay. The sprouted legumes were also investigated for their Vitamin C content and total carotenoid content. The results revealed that 60% EHWE was best suited for extraction of TPC for T_3 and T_4 while 60% MHWE was better suited for T_1 and T_2 while 60% EHWE exhibited highest DPPH radical scavenging activities and reducing power assay for T_4, T_3 and T_2 while 60% MHWE proved to be a better solvent for T_1 while 60% MHWE exhibited highest ABTS scavenging activities for T_1, T_3 and T_4. HWE was the least potent amongst all the solvent systems tested. T_4 showed the highest TPC and antioxidant activities although no correlation was obtained between the TPC and antioxidant activities. The results of the study reveal that solvents with different polarity had significant effects on TPC and in-vitro antioxidant activities. This information may be of interest to the food industry as legumes are cheap sources of nutrients, bioactive compounds and sprouting is an inexpensive technique to improve their functional value which can be exploited for prevention of lipid oxidation in food systems.

Introduction

Over the past years interest in natural antioxidants for improvement of food stabilization has in natural antioxidants for improved food stabilization has intensified. For the food industry, moving to antioxidants from natural sources is a potential goldmine that offers a "green" label for food stabilizers. Thus, evaluation of antioxidant capacity in vitro and/ or in some food model system has thus become a very active topic for research for screening of potent natural antioxidants, and an alphabet soup of assays has evolved to identify these nature gifted materials and identify the likely candidates that have the potential to be used in food preservation industry.

Legumes have been consumed as a part of the Indian diet since ages and are considered to be functional foods due to their nutritive value as well as presence of bioactive compounds. Further, germination is an inexpensive and effective technology for improving the quality of legumes by enhancing their functional value. Germination causes important changes in the biochemical, nutritional and sensory characteristics of seeds. Sprouts, in addition of being good source of basic nutrients, contain important health promoting phytochemicals such as phenolic compounds (Troszynska *et al.,* 2002), which increase during germination process and are therefore considered to fulfill several functions, act as reducing agents, terminators of free radicals, metal chelators and singlet oxygen quenchers. They have been long established to possess health promoting activities such as reduction of risk of cancer, heart disease, diabetes, inhibition of plasma platelet aggregation, cyclooxygenase activity, and release of histamine, in vitro antibacterial, antiviral, anti-inflammatory and anti-allergenic activities (Oak *et al.,* 2005). Apart from these functions they can also prevent oxidation in foods and act as shields against oxidative damage in human body (Kikuzaki *et al.,* 2002). As a result of this activity, the presence of phenolic compounds in food has in the recent years come to be viewed in a positive light by researchers as well as the consumers and hence resulted in a push to procure food with beneficial effects. The benefits towards many of these conditions come in part through the antioxidant characteristic of phenols, hence making it relatively important to quantify, identify and evaluate their antioxidant activities. The addition of antioxidants has become popular as a means of increasing the storage period of food products and improving the stability of lipids and lipid-containing foods without loss of sensory and nutritional qualities. Elimination of synthetic anti-oxidants in food applications has given more impetus to exploring natural sources of anti-oxidants assuming that natural compounds are safer, prefer natural antioxidants to synthetic antioxidants. Sprouts, are assumed to be good sources of these natural antioxidants and hence can be exploited in food, pharmaceutical and cosmetic industry. Therefore, the present study was conducted to elucidate the effect of extraction with different solvents on total phenolic compounds and antioxidant activity of sprouted legume seeds commonly consumed in India so that they could use as functional compounds to enhance the shelf life of easily perishable foods especially of livestock based origin

Materials and Methods

Sprouting of legume seeds

Legume seeds (Mung gram-T_1, black gram-T_2, Pigeon pea- T_3 and Cowpea-T_4) were purchased from M/s Pathak Bheej Bhandar, Bareilly, UP. After cleaning of the extraneous material seeds were washed and soaked in water for 12 hrs. The excess water was thereafter drained off and seeds were sprouted

in jars covered with muslin cloth. The seeds were rinsed periodically and water drained off. Sprouted beans 3-4 days old were collected, dried in a food drier for 18 h, ground to fine powder, sieved and stored in PET containers for further analysis.

Solvent extraction

The ground flour was extracted using three different solvents [(hot water (HWE), ethanol: hot water (60:40) (EHWE), methanol: hot water (60:40) (MHWE)]. About 20 g of flour was extracted in 100 ml of solvent for 24 h in an orbital shaker at room temperature. The residues were re-extracted with 100 ml of the solvent for additional 8h. The supernatants collected were pooled and centrifuged at 8000 rpm for 15 minutes, supernatants were filtered through a Whatman no 2 filter paper. The filtrate was then evaporated at 40 C in a rotary evaporator. The dried material were redissolved in the same solvent to a concentration of 250 mg/ml (stock solutions) and stored at -20 C till further analysis. The stock solution was further diluted to obtain various concentrations at the time of analysis.

Determination of Total Phenolic Content (TPC)

The TPC was determined as per the procedure of Singelton and Rossi, (1965) with slight modifications. An aliquot of 100 µl of extracts were mixed with 0.75 ml of Folin Ciocalteu reagent to make the volume ten times (8.5 ml) with distilled water and allowed to stand at room temperature for 5 min. Thereafter, 0.75 ml of 6% sodium bicarbonate was added to it and incubated at room temperature for 90 min in dark and the absorbance was measured at 765 nm. A standard curve was plotted using different concentrations of gallic acid (0.05, 0.1, 0.15 and 0.2 mg/ml) and the amount of TPC was calculated as gallic acid equivalents (GAE) in mg/g of dried plant powder.

DPPH radical scavenging activity

Different concentration such as 0.2, 0.4, 0.6, 0.8 and 1.0 ml (1mg/ml) legume extract was taken in test tube and its volume was made upto 4 ml with distilled water (Fargere *et al.*, 1995). 1 ml of 1 mM DPPH (1 mM DPPH methanolic solution was prepared) was added. All test tubes were shaken well and allowed to stand at room temperature for 30 min. Control was prepared by adding 1 ml DPPH solution and a volume of 4 ml DW, absorbance was immediately taken at 517 nm. DPPH activity as percent inhibition was calculated using the following formula

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

ABTS radical scavenging activity

ABTS stock solution was prepared as per the method of Re *et al.*, (1995). Prior to use the ABTS stock solution was diluted to an absorbance of 0.70± 0.02 with ethanol. About 4.9 ml of ABTS diluted solution was added to 100 µl of plant extract and the absorbance measured after 20 minutes at 734 nm using ethanol as blank

ABTS activity (% inhibition) = [(0.7-At20)/0.7] x 100

Reducing power assay

The reducing power of the extracts was determined according to the method of Oyaizu, (1986). Different concentrations of the legume extracts were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide in 10 ml test tubes. Then the mixture were incubated for 20 min at 50 ^{0}C followed by the addition of 2.5 ml of 10 % trichloroacetic acid,

centrifuged (700 x g /10 min). After that 2.5 ml supernatant was mixed with 2.5 ml of DW and 0.5 ml of ferric chloride (0.1% w/v) and absorbance was measured at 700 nm.

Total carotenoid content

The carotenoids were extracted by the procedure of Zakaria *et al.,* (1979) and the carotenoid content was obtained by measuring the absorbance at 480 nm in a UV-Visible spectrophotometer and calculated using the formula

Amount of carotenoids (mg/g) = A_{450} x volume of the sample x 100 x 4/ weight of the sample

Vitamin C content

The Vitamin C (Vc) content was estimated using the method described by method of Osborne and Boggt, (1978) with slight modifications using the 2,6-dichloroindophenol titrimetric method.

Statistical analysis

The data obtained from the analysis was pooled and analyzed using SAS (Statistical Analysis Software) (version 9.3, SAS Institute Inc. Cary, NC, USA). Means and standard error were computed for each parameter. The data were subjected to analysis of variance, least significant difference and Tukey test for comparing the means.

Results and Discussion

Total phenolic content

Phenolic compounds are secondary metabolites in plants possessing an aromatic ring bearing one or more hydroxy substituents including flavonoids (flavones, isoflavones and flavonones), anthocyanins and catechins (Rice Evans *et al.,* 1997). Natural phenolics are mainly known to exert their beneficial health effects through their antioxidant activity (Fang *et al.,* 2002). These compounds are capable of acting as hydrogen or electron donors which can stabilize and delocalize the unpaired electron, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases (Heim *et al.,* 2002). TPC of plant very much depends on parts of the plant as they are unevenly distributed in plant parts used for extraction and the type of solvent used which have antioxidant effects. Among the various legumes tested the highest phenolic content (Table 1) was recorded in the following order T4 >T1> T2> T3. A significant ($p<0.05$) difference was observed in between the different sprouted legumes. Sreeramulu *et al.,* (2009) reported that pulses and legumes had a TPC that ranged from 62.35 to 418.34 mg/100 g while Marathe *et al.,* (2011) reported a TPC range of 0.325-6.378 mg/g GAE in various legumes with the highest concentration being recorded in cowpea (red and brown). Siddhuraja and Becker, (2007) observed high TPC in cowpea extracts which varied from 64-173 mg TAE/ g while Sreerama *et al.,* (2012) observed a TPC of 12.16 mg GAE/g in the same. Ojwang *et al.,* (2013) and Awika and Duodu, (2017) reported cowpeas to have a unique profile of some polyphenols especially phenolic acid derivatives, flavanol glycosides, anthocyanins, flavan-3-ols (tannins) especially catechin-7-O-glucoside, which provides bioactive properties that are unique in nature.

The type of solvents used for extraction had significant ($p<0.05$) effect on effectiveness in extracting phenolics from plant components. HWE proved to be the least potential in extraction of polyphenolic compounds from sprouted legume amongst the tested solvent systems. The TPC value was affected by the extracting solvents in the following order from high to low: EHWE > MHWE > HWE for

$T_3$and T_4 and MHWE> EHWE > HWE for T_1 and T_2. The results suggested that EHWE was the best solvent for extraction of phenolic compounds from T_3 and T_4 whereas MHWE was better extraction solvent for T_1 and T_2. Overall, it was observed that EHWE served the purpose of a better solvent for extraction of phenolic compounds from legumes and T_4 had highest TPC among tested legumes.

Amongst interactions involved TPC was significantly ($p<0.05$) highest in EHWE of T_4 followed by MHWE and HWE extracts which varied significantly ($P<0.05$) amongst themselves. This order was followed by MHWE and EHWE of T_1 amongst which a significant ($P<0.05$) variation was observed. The substantial variability in the results obtained may be influenced by many complex factors which includes phenotype, crop location, weather conditions, environmental stress, post-harvest environments, difference in extraction solvent (polarity), stage of germination and photoperiods, degree of polymerization of phenolic compounds, interaction of phenolics with other food constituents, time and temperature used for extraction. Thus, there is no uniform or completely satisfactory procedure suitable for extraction of all phenolic compounds from plant materials (Naczka and Shahidi, 2004).

DPPH radical scavenging activity

The reduction of DPPH radical is a measure of antioxidant activity (Thippeswamy and Naidu 2005). Upon receiving a proton from any hydrogen donor mainly phenolics, DPPH loses its chromophore and becomes yellow in colour. As the degree of hydroxylation increases, the DPPH radical scavenging activity and the antioxidant activity of the compound increases. The highest activity was recorded in T_4 which ranged from (38.66 to 56.90 %) followed by T_1 (28.97 to 43.85%) and T_3 (26.07- 41.15%) although no significant differences were observed between T_1 and T_3. The lowest activity was recorded in T_2 (18.96- 31.81%). The strong antioxidant activity of cowpeas may be explained by their high phenolic content. As per the observations of the present study (Table 2) and available literature it is evident that antioxidant activity of sprouted legumes were significantly ($p<0.05$) affected the type of solvent used. EHWE exhibited greater DPPH scavenging activity for T_4, T_3 and T_2 while MHWE proved to be a better solvent for T_1. Amongst the whole set of interactions tested T_4EHWE had significantly ($p<0.05$) higher activity followed by T_4MHWE and T_1MHWE. The interactions between T_3MHWE, T_1EHWE and T_4HWE did not show significant ($p>0.05$) differences although they showed significantly higher inhibition as compared to T_2EHWE, T_2MHWE, T_1HWE. The lowest activities were observed in T_3HWE and T_2HWE and their activities were significantly ($p<0.05$) lower than all other treatments. Marathe *et al.*, (2011) categorized cowpea (brown) under legumes showing high DPPH radical scavenging activity (>400 units/g) as compared to the other three legumes that showed intermediate activity towards scavenging of DPPH radicals. Similarly, high radical scavenging activities of cowpea extracts have been noted by Siddhuraju and Becker, (2007) and Sreerama *et al.*, (2012). High concentrations of protocatechuic acid contributing to the hydrogen donating ability may be the reason behind the greater efficacy of cowpea extracts as antioxidants (Yen *et al.*, 2005)

ABTS activity

The ABTS assay quantifies an antioxidant (hydrogen/ electron donor) suppression of the radical cation based on single electron reduction of the relatively stable $ABTS^+$ cation produced formerly by an oxidation reaction.

Table.1 Comparison of effects of different extraction solvents on total phenolic content (TPC) of sprouted legumes (Mean±S.E)

Treatment	HWE	EHWE	MHWE	Treatment Mean
T1	2.178± 0.106^{bC}	5.066 ± 0.114^{bB}	6.092±0.110^{bA}	4.445± 0.406
T_2	1.762±0.100^{bB}	2.851±0.128^{cA}	3.015±0.084^{cA}	2.543± 0.146
T_3	0.773±0.050^{cB}	2.236±0.051^{cA}	2.015±0.072^{dA}	1.675±0.159
T_4	6.476±0.056^{aC}	12.647±0.156^{aA}	10.112±0.151^{aB}	9.745±0.618

Where, T_1-mung gram, T_2- black gram, T_3- pigeon pea, T_4- cowpea (brown),HWE- Hot water extract, EHWE- Ethanol: Hot water (60:40), MHWE- Methanol: Hot water (60:40). Mean ±S.E. with different superscripts row wise (capital alphabet) and column wise (small alphabet) differ significantly ($p<0.05$), n=6 for each treatment

Table.2 Comparison of effects of different extraction solvents on DPPH scavenging activity (DPPH) of sprouted legumes (Mean±S.E)

Treatment	HWE	EHWE	MHWE	Treatment Mean
T_1	28.97±2.13^{abB}	38.80±2.17^{bcAB}	43.85±2.44^{abA}	37.20±1.16^{b}
T_2	18.96±1.60^{bB}	31.81±1.88D^{bcA}	30.45±1.72^{bcA}	27.07±1.59^{c}
T_3	26.07±1.95^{bB}	41.15±2.32^{bA}	38.80±2.09^{bA}	35.34±1.40^{b}
T_4	38.66±2.07^{aB}	56.90±2.57^{aA}	52.52±2.49^{aA}	49.36±2.18^{a}

Where, T_1-mung gram, T_2- black gram, T_3- pigeon pea, T_4- cowpea (brown),HWE- Hot water extract, EHWE- Ethanol: Hot water (60:40), MHWE- Methanol: Hot water (60:40).
Mean ±S.E. with different superscripts row wise (capital alphabet) and column wise (small alphabet) differ significantly ($p<0.05$), n=6 for each treatment

Table.3 Comparison of effects of different extraction solvents on ABTS scavenging activity (ABTS) of sprouted legumes (Mean±S.E)

Treatment	HWE	EHWE	MHWE	Treatment Mean
T_1	23.06±2.55^{aB}	43.91±2.83^{abA}	48.14±3.07^{aA}	38.37±1.99^{a}
T_2	16.13±1.89$^{a\ B}$	35.37±4.04^{abcdA}	31.72±3.32^{bAB}	27.74±2.03^{b}
T_3	20.98±2.13^{aB}	41.21±3.60^{abcA}	44.57±3.32^{abA}	35.59±2.08^{a}
T_4	25.64±2.66^{aB}	51.35±3.20^{aA}	53.99±3.18$^{a\ A}$	43.66±2.19^{a}

Where, T_1-mung gram, T_2- black gram, T_3- pigeon pea, T_4- cowpea (brown), HWE- Hot water extract, EHWE- Ethanol: Hot water (60:40), MHWE- Methanol: Hot water (60:40).
Mean ±S.E. with different superscripts row wise (capital alphabet) and column wise (small alphabet) differ significantly ($p<0.05$), n=6 for each treatment

Table.4 Comparison of effects of different extraction solvents on reducing power assay of sprouted legumes (Mean±S.E)

Treatment	HWE	EHWE	MHWE	Treatment Mean
T1	0.285±0.04	0.353±0.05^{b}	0.412±0.05^{b}	0.392±0.03^{b}
T2	0.214±0.03	0.305±0.04^{b}	0.293±0.04^{b}	0.271±0.02^{c}
T3	0.289±0.04	0.458±0.06^{ab}	0.428±0.06^{ab}	0.350±0.03^{b}
T4	0.395±0.05^{B}	0.659±0.08^{aA}	0.570±0.07^{aAB}	0.541±0.04^{a}

Where, T_1-mung gram, T_2- black gram, T_3- pigeon pea, T_4- cowpea (brown),HWE- Hot water extract, EHWE- Ethanol: Hot water (60:40), MHWE- Methanol: Hot water (60:40).
Mean ±S.E. with different superscripts row wise (capital alphabet) and column wise (small alphabet) differ significantly ($p<0.05$), n=6 for each treatment

Table.5 Comparison of Vitamin C content in various sprouted legumes (Mean±S.E)

Treatment	Raw seeds	Germinated seeds (mg/100g FW)
T1	ND	19.632±0.392[b]
T2	ND	36.688±0.735 [a]
T3	ND	4.635±0.179[c]
T4	ND	18.725±0.272[b]

Where, T_1-mung gram, T_2- black gram, T_3- pigeon pea, T_4- cowpea (brown). Mean ±S.E. with different superscripts column wise (small alphabet) differ significantly ($p<0.05$), ND- not detected, n=6 for each treatment

Table.6 Total carotenoid content (µg/100g) of various sprouted legumes

Treatment	Total carotenoid content (µg/100g)
T_1	311±11.38[d]
T_2	102.5±4.94[c]
T_3	923.33±8.47[b]
T_4	2903.33±26.61[a]

Where, T_1-mung gram, T_2- black gram, T_3- pigeon pea, T_4- cowpea (brown). Mean ±S.E. with different superscripts column wise (small alphabet) differ significantly ($p<0.05$), n=6 for each treatment

Perusal of Table 3 revealed that the highest activity was observed in T_4 followed by T_1, T_3 and T_2. The ABTS inhibitory activity did not significantly ($p<0.05$) differ among treatments except for T_2. Amongst the different solvents it was observed that EHWE extracts of T_4 and T_2 showed higher inhibitory activity as compared to their counterparts while MHWE extracts of T_1, T_3 were found to be more inhibitory toward the ABTS cation. Nonetheless, the highest inhibition was observed in T_4EHWE followed by T_4MHWE and T_1MHWE although their activities did not vary significantly ($p>0.05$) from each other. T_3MHWE and T_1EHWE possessed moderate inhibitory activity followed by T_3EHWE and T_2EHWE.

A significant ($p<0.05$) decrease in activities were observed in T_2MHWE and T_4HWE while the lowest inhibitory activities were observed in HWE. 60% ethanol and 60% methanol served to be better solvents for extraction of active components from legume extracts as compared to their aqueous counterparts. Marathe *et al.,* (2011) observed highest ABTS activity in brown cowpea extracts among various legumes while Xue *et al.,* (2016) reported significantly higher activities in mung bean sprouts as compared to black bean and soyabean sprouts. Hagerman *et al.,* (1998) explained that high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS+) and their effectiveness depends on molecular weight, presence of aromatic rings and nature of hydroxyl groups substitution than the specific functional groups.

Reducing Power Assay (RPA)

Reducing ability reactions involve a single electron or hydrogen transfer mechanism, where the predominant phenolic functional groups reduce the oxidized metabolites of peroxidation. Perusal of Table 4 revealed that cowpea showed the highest RPA amongst the four tested legumes. The reducing power of legume extracts from different solvent extractions differed significantly ($p<0.05$) from each other. The RPA was affected by the extraction solvents in the following ascending

order of 60% MHWE > 60% EHWE > HWE for mung bean (T_1), while 60% EHWE proved to better extraction solvent for black gram (T_2), pigeon pea (T_3) and cowpea (T_4). The highly pigmented cowpeas possessed relatively higher ($p<0.05$) RPA as compared to the other legumes with the lowest activity in T_2. Intermediate RPA was observed in T_3 and T_1 and did not differ significantly ($p>0.05$) from each other.

Amongst the various legume extracts tested the highest significant ($p<0.05$) activity was observed in T_4EHWE followed by T_4MHWE and T_3EHWE. The ranking in antioxidant activity was followed by T_3EHWE and T_3MHWE which did not vary significantly ($p>0.05$) from each other but showed higher activities than T_1MHWE, T_4EHWE and T_1EHWE. The lowest activities were observed in HWE of T_3, T_1 and T_2 which can be strongly correlated to their lower phenolic content.

Similarly Sreerama *et al.,* (2012) reported cowpea extracts to have higher FRAP as compared to horse gram and chick pea extracts while Xue *et al.,* (2016) reported mung gram extracts to have higher RPA as compared to soyabean and black beans during progress of germination. The higher RPA of the cowpea extracts may be due to the presence of isoflavones (daidzein and genistein) (Mazur *et al.,* 1998) or may be due to presence of electron donors such as polyphenols and carotenoids which may reduce Fe^{3+}/ ferricyanide to ferrous form (Chung *et al.,* 2002). Duenas *et al.,* (2005) revealed cowpea flour was considered as an antioxidant due to its high antioxidant activity. Similar positive correlations between the phenolic content and phospomolybedenum activity, ferric reducing antioxidant power by Sowndhararajan *et al.,* (2011), Boateng *et al.,* (2008) and Marathe *et al.,* (2011) in commonly consumed legumes.

Vitamin C content

Vitamin C (Vc) functions as an antioxidant and minimizes free radical damage in cells. Vc was almost absent in raw legume seeds but is however synthesized during the germination process (Khyade *et al.,* 2016). The highest Vc content was observed in T_2 followed by T_1 and T_4 while the lowest content of Vc was recorded in T_3 samples (Table 5). No significant ($p>0.05$) difference was observed between T_1 and T_4 where the Vc was 19.63 and 18.72 mg/100g FW resp. Remarkable increase in Vc contents of mung beans sprouts have been reported by Gan *et al.,* (2016) while Xue *et al.,* (2016) observed higher Vc content in black beans as compared to mung bean at different stages of germination although the mechanism of ascorbic acid production in sprouts has not yet been clarified. The increase in Vc level may be considered to be a consequence of the reactivation of ascorbic acid biosynthesis undergone in the seeds during germination (Mao *et al.,* 2005).

Total carotenoid content

Carotenoids may act as singlet oxygen quencher and transfer an electron to the radicals thereby giving rise to stable carotenoid radical cation generating the original molecule (Mortensen and Skkibsted, 1997) (Table 6). A wide variation was observed in the carotenoid content of sprouted legume seeds studied ranging from 102.5±4.94 (µg/100g) in T_2 to 2903.333±26.61 (µg/100g) in T_4. Upon germination the concentration of β-carotene steadily increases with increasing germination time. High concentration (44 mg/ 100 g DW) of total carotenoids have been reported by Mosha *et al.,* (2009) in cowpea leaves while Kandlakunta *et al.,* (2008) reported pulses to be a better source of carotenoids as compared to cereals. The appreciably high amount of

carotenoids in the legumes tested in the present study suggests that these compounds can be used as antioxidants. Choi *et al.*, (2007) tested various commonly used grains and reported the highest carotenoid content in mungbean (102 µg/100g) and lowest content in white rice (1 µg/100g).

On the whole the study indicated that sprouted legumes are natural sources of bioactive compounds and antioxidants. The results indicated that significantly different phenolic contents and antioxidant activities were extracted by different solvent systems. Among the different solvents 60% ethanol was the best solvent for the best antioxidant activity and among the different solvents tested T_4 (cowpea) exhibited highest phenolic content and antioxidant activity. This study provides the evidence that the germinated seeds are valuable sources of natural bioactive compounds and antioxidants and hence have the potential to provide health benefits as well improve the shelf stability of foods.

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How to cite this article:

Serlene Tomar, Geeta Chauhan, Annada Das and Med Ram Verma. 2018. Comparative Evaluation on Phenolic Content and Antioxidant Activity of Legume Sprouts as Affected by Various Solvents for Application in Livestock Products. *Int.J.Curr.Microbiol.App.Sci.* 7(05): 3388-3398. doi: https://doi.org/10.20546/ijcmas.2018.705.396