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Characterization of Phenolic Content and Antioxidant Properties of Soybean Seeds

Chitra Gupta[1], Ravi Kant Kannaujia[1], Rohit Verma[1], Rabia Kouser[2] and Mohd. Asif[2]

[1]Deptt. of Chemistry , Bundelkhand University Jhansi (U.P.) 284128
[2]Deptt. of Chemistry,Awadhesh Pratap Singh University, Reewa (M.P.) 486003
Email: chitra.jhansi@rediffmail.com, ravikkannaujia@gmail.com, rohitjhansi_20@rediffmail.com, b.h.khan92@gmail.com, malik.asifprobable@gmail.com

Abstract

Antioxidant activity, free radical scavenging activity and Phenolic compounds play also an important roll in plant resistance and defence against microbial injection which are intimately connected with reactive oxygen species (ROS). Together oil and protein content account for about 60% of dry soybeans by weight; protein at 40% and oil at 20%. The remainder consists of 35% carbohydrate and about 5% ash. Soybean cultivars comprise approximately 8% seed coat or hull, 90% cotyledons and 2% hypocotyls axis or germ.the radical scavenging activity (RAS) was soybean seeds. The study indicated that soybean possessed the highest antioxidant capacity and phenolic compounds concentration among all the soybean seed studied. In this paper multiresidue determination of phenolic content & antioxidant properties of soybean seed are discussed using DPPH,HPLC.

Keywords : Antioxidant activity , HPLC , DPPH, Phenolics, Soybean Seed

INTRODUCTION

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that .oxidation reaction can produce free radicals. These free radicals are highly reactive oxygen molecules containing species. And include superoxide and hydroxyl radical peroxyl radical and peroxy nitrile radicals. Antioxidants terminate these chain reactions by removing these free radicals. Intermediates and inhibit other oxidation reaction they do this by being oxidized themselves so antioxidation are often reducing agent such as thiols, ascorbic acid or

polyphones. The most reactive and damaging free radicals are the hydroxyl radical and peroxyl radical. Hydroxyl radical is particularly unstable and will react rapidly with most of biological molecules and can damage cells.[1] By starting chemical chain reaction such as lipid per oxidation by oxidation DNA or proteins.

Although oxidation reactions are crucial for life. They can also be damaging hence plant and animal maintain complex system of multiplayer of antioxidant , such as glutathione, vitamin C and vitamin E as well as enzymes such as catalyse, superoxidant or inhibition of the antioxidedant enzymes cause oxidation stress and may damage or kill cells.

Antioxidants are believed to play a very important role in the defence system against reactive oxygen species.[2] Because of its ability to scavenge free radicals i.e. they acts as free radicals scavenge and pair with free radicals changing them harmful to non species although mitochondria is main source of free radical. There are numerous other sources which released free radical.

These free radicals may oxidize nucleic acid, protein, lipids and DNA can initiate degenerative disease. Although antioxidants can not completely rid our bodies from free radicals. They block the process of oxidation by neutralizing free radicals by neutralizing themselves became oxidized.

So antioxidant compounds are playing an important role as a health protection factor. The main characteristic of an antioxidant is its ability to trap free radical. An antioxidants compound like Phenolic acid, Polyphones and Flavonoides scavenge free radical such as peroxide hydroperoxide or lipid peroxyl and thus inhibits the oxidative mechanisms that led to degeneration disease.

Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health diseases and even altitude sickness, although initial studies suggested that antioxidant supplement might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. [2, 3] In addition to these uses of natural antioxidant in medicine these compounds are also useful in cosmetics and preventing the degradation of rubber and gasoline.

Naturally occurring plant phenolics include several groups of compound that have health promoting properties. Phenolics may act as antioxidants. There by reducing the risk of atherosclerosis and coronary heart disease, this can be caused by oxidation of low lipoproteins. They also may protect against some form of cancer.[4] Depending on the substituents of a phenolic hydroxyl group their antioxidant properties comprise all know mechanisms this is of important oxidative stress. Many phenolic compounds found in plant tissues are potential antioxidants: Flavonoids, tannins and lignin precursors may all work ROS scavenging compound. Antioxidant acts as a co-operative network, employing a series of different redox reaction interaction between ascorbic acid and glutathione and ascorbic acid and phenolic compound are well know. [5] To prevent oxidation of fat and oils. Antioxidants are widely used in food and cosmetics. Because of possible toxicity of the widely used butylated hydroxyl toluene (BHT) and butylated hydroxyanisol (BHA) together with consumers. Preference for natural product, much research on natural antioxidants has been undertaken in the recent post. [6]

MATERIALS & METHODS

All solvents / chemical used were analytical/BDR grade. DPPH was obtained from March, Mumbai, India. Soybean seed were purchased from local market in Jhansi U.P.

UV – Vis. Spectrophotometer:

UV – Visual measurement were performed on a UV–Visible 1700 spectrophotometer SHIMADZU.

(A) Physico-Chemical analysis

Sampling:

Soybean treatments were used for all analysis.

Acidity:

The acidity was measured by titration with o.1 N NaOH to Ph 8.1 and expressed as malic acid. Acidity was expressed as % (g/100g). [159]

pH:

10 g of samples were homogenized for pH measurements. A digital pH meter was employed at 25°C

Total soluble solids:

The content of total soluble solids was determined using samples of fruit pulp with a hand refract meter, at room temperature (range from 18 to 23°C)

Ratio (solids/acidity)

The ratio was calculated using the relation between the total soluble solids by acidity.

Tannin:

Tannin content was determined according to **Horwitz**. [7]

(B) Determination of Total Phenolic Contents:

This is a non destructive; rapid and sensitive which can detect a very small quantity following three different methods have been used for the determination of phenolic content which was calculated from the standard graph.

1. Prussian -Blue method
2. Butanol-HCl Method
3. Vanillin-HCl Method

(C) Antioxidant Activity:

1, 1- diphenyl-2-picrylthydrarzil (DPPH) assay method.

(B) Determination of Total Phenolic Contents

1. Prussian Blue Method:

This method has been described by **Vinson**[8] and **prior**[9,10] for the determination of total phenolics

Reagent:
0.5 M $FeCl_3$ in 0.1 N HCl
0.008 M K_3 [Fe $(CN)_6$]
100% methanol solution

Principle:
Ferric ion (Fe^{3+}) is reduced to ferrous ion (Fe^{2+}) by Tannin and other phenolics to form a Ferric – Cyanide, Ferrous ion color complex commonly known as Prussian Blue,

Plant Material:
Healthy and fresh Soybean seed were selected and purchased from the local market at Jhansi and also collected naturally.

Preparation of Samples
Soybean seed was cleaned under running tap water excessive water was drained off. The soybean seeds were cut into small pieces and subjected to size reduction using kitchen blender with a kitchen mixer to get a thick paste, and kept at 20^oC for further analysis.

Preparation of Extract [9]
250 mg of plant sample was extracted with 10 ml of 100 % methanol and left it over night, Next day filtered with whatman filter paper and make up the volume up to 25 ml with 100% methanol.

Procedure
Take 0.1 ml of aliquot was taken. It was diluted within 60 ml of distilled water and added 3 ml of 0.5M $FeCl_3$ in 0.1N HCl and 3 ml of 0.008M $K_3[Fe(CN)_6]$. Colour developed immediately after 10-15 min.

Spectrophotometric Measurement:
U.V.Spectrophotometer is used for the determination of optical density "after leaving the prepared sample solution for 10-15 minutes record the absorbance of sample against blank at 725 nm". The optical density of the above solution was determined at 725 nm.
A blank of identical composition but omitting the extract was analyzed and subtracted from all other readings. The phenolic content can be calculated by using the following formula [9,11]

$$\% \text{ Phenolic content} = \frac{\text{OD x Factor x 25 x 100}}{\text{Conc. x wt. of sample}}$$

Tannic acid was used as the standard, and the Total Polyphenol content was expressed as Tannic acid equivalent (mg/ml)
Standard solution of Tannic acid:
Standard solution of tannic acid is prepared by taking the 10 mg of tannic acid and

dissolved in 100 ml distilled water than take 10 ml of this solution in another volumetric flask of 100 ml and make the final volume up to 100 ml by adding distilled water and now from volumetric flask containing 0.01 mg/ml of tannic acid make different solution.

Spectrophotometric Measurement:
Spetrophotometer is used for the determination of optical density the 3 ml of 0.5M $FeCl_3$ in 0.1N HCl was added to the .001, .002, .003, .004, …….. 0.010 ml 0.012 ml of tannic acid followed immediately by timed addition of 3 ml of 0.008 M $K_3[Fe(CN)_6]$, but omitting the extract and using distilled water is different volume used (Final volume in 60 ml). After waiting for 15-20 min take the reading and now record the absorbance at 725 nm wavelength in spectrophotometer of the different solution.

General Aspects of the Prussian blue test
A large access of $FeCl_3$ is added in all soybean seed solution of the test. This is to ensure rapid and complete reaction. The blank is kept low by using low $K_3[Fe(CN)_6]$ concentrations. The absorbance increases with time. Formation of the complex appears to be rate limiting, the redox reaction apparently reaching completion in second.

Because of inherent instability of dilute solution of $FeCl_3$, this reagent is not added until just before the solutions are ready to be analyzed. The results do not be seemed to change noticeably, when $FeCl_3$ is added up to half an hour before analysis of the samples as long as no organic solvent are present. A solution containing 3 ml of methanol in 60ml of water however showed a 30% increase in absorbance of the blank when the Iron was added 30 min. early. In the different concentration of Prussian blue $K_3[Fe(CN)_6]$ solution was changed. Although the absorbance due to tannin was not affected, this increase in background requires that ferric chloride be added at time intervals when organic solvent are present. All dilution was made from 0.1M $FeCl_3$ in 0.1N HCl (added to increase stability). This stock solution is stable for months.

The A_{725} reading is extremely sensitive to the slit width of the spectrophotometer. Widening the slit width from 0.08 mm to near maximum (0.2mm) reduced the absorbance by 46%.

Butanol –HCl Method
This method has been used for the determination of Tannin.

Reagent:
Butanol- HCl (95:5)
Ferric reagent (2% Ferric ammonium sulphate in 2NHCl)
70% aqua Acetone solution.

Principle:
The Butanol- HCl method originally proposed for measurement of condensed tannin. This method yields pink colored anthocyanidins or oxidative cleavage of the inter

flavan bonds of condensed Tannin. This method can only be used to compare chemically similar Tannin since reactivity in the Butanol- HCl assay in the function of the interflavan bonds. This method therefore measured relative degree of polymerization and has been used to investigate nature of condensed Tannin.

Plant Material:
Healthy and fresh Soybean seedwere selected and purchased from the local market at Jhansi and also collected naturally.

Preparation of Samples
Soybean seed was cleaned under running tap water excessive water was drained off. The soybean seeds were cut into small pieces and subjected to size reduction using kitchen blender with a kitchen mixer to get a thick paste, and kept at 20^{o} C for further analysis.

Preparation of extract [12]
250 mg of plant sample was extracted with 10 ml of 70 % aq. Acetone and left it over night. Next day filtered with whatman filter paper and make up the volume up to 25 ml with 70 % aq. Acetone.

Procedure
0.5 ml of aliquot was added in 3 ml of Butanol: HCl (95:5) solution and 0.1 ml of ferric reagent in a 100 ml conical flask. Heat this solution in water bath for 1 Hour, a pink colour developed. Leave it for 10 to 20 minutes.

Spectrophotometric Measurement:
U.V. Spectrophotometer is used for the determination of optical density. After leaving the prepared sample solution for 10-15 minutes record the absorbance of sample against blank at 550 nm. The optical density read at 550nm in spectrophotometer which has been zeroed with water. A blank of identical composition but omitting the extract was analyzed and subtracted from all other readings. The Tannin contain can be calculated by using the following formula.

$$\% \text{ Tannin contain} = \frac{\text{OD} \times 78.26 \times \text{Dillution factor}}{(\% \text{ Dry Matter})}$$

VANILLIN –HCl Method
This method has been described by **Buttner** and **Burns[13]** for the determination of condensed Tannin.

Reagent:

4% vanillin in methanol
8% HCl in methanol
70% aqueous acetone

Principle
Vainillin test is used quantification of condensed Tannin. The Vanillin test is specific for flavan 3- Ols di hydrochalocones and proandthocyanins.

Plant Material:
Healthy and fresh Soybean seed were selected and purchased from the local market at Jhansi and also collected naturally.

Preparation of Samples
Soybean seed was cleaned under running tap water excessive water was drained off. The soybean seeds were cut into small pieces and subjected to size reduction using kitchen blender with a kitchen mixer to get a thick paste, and kept at 20^{o} C for further analysis.

Preparation of Extract [9]
250 mg of plant sample was extracted with 10 ml of 70 % aq. Acetone and left it over night. Next day filtered with whatman filter paper and make up the volumes up to 25 ml with 70 % aq. Acetone.

Procedure:
The Vanillin Test was performed as described by **Burns (1971)[14]** For the corrected Vanillin test (see results) separate blanks were read for each sample an subtracted from the result of the regular vanillin test. The blank were run under condition identical with the regular Vanillin test except that Vanillin was omitted from the 8% HCl in methanol. 1ml of aliquot were added in 5 ml of 4% Vanillin and 5 ml of 8% HCl in methanol. Leave if for 5-10 min.

Spectrophotopemetric Measurement:
U.V. spectrophotometer is used for the determination of optical density. After leaving the prepared sample solution for 10-15 minutes record the absorbance of sample against blank at 500 nm. The optical density read at 500 nm in spectrophotometer which has been zeroed with water.
A blank of identical composition but omitting the extract was analyzed and subtracted from all other reading. The condensed Tannin can be calculated by using the following formula [15,16]:

$$\% \text{ Condensed Tannin} = \frac{\text{OD x Factor x 25 x 100}}{\text{Conc. x wt. of sample (mg)}}$$

Standard For Vanillin- HCL Method :
Catechin or Tannic acid is used for making standard curve of Vanillin- HCl method. Tannic acid solution of 2000 pm were prepared by dissolve 10 mg. Tannic acid in 5 ml methanol make different ppm solution. 11 conical flask of 25 ml were taken. They were marked up to T, T_1 to T_{10} in T no Tannic acid were added and 1 ml of each pm solution 5 ml of (4 % Vanillin +8 % HCl solution) a light chocolate or yellowish brown colour developed. Leave it for 15-20 min and record the reading at 500 nm against blank containing 1 ml distilled water + 5 ml (4 % Vanillin + 8 % HCl solution).

(C) Antioxidant activity:

Evaluation of the Free Radical Scavenging Activity (FRSA) in the 1,1-diphenyl-2-picryl hydrazil radical (DPPH) assay:

Radical Scavenging Activity (RSA) of the extracts was determined by DPPH method as described by **Blois [17] (1958)**; **Pierori et al[18] (2002)** and **Prior et al[9,10]**

Reagent:

0.1 ml methanolic DPPH solution
100% methanol solution

Principle:

A sample method that has been developed to determine the antioxidant activity of foods utilizes the stable 2,2- dipenyl -1- picrylhydrazyl (DPPH) radical the structure of DPPH and its reduction by an antioxidant are shown above. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow as the absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourization is stoichiometric with respect to number of elections captured.

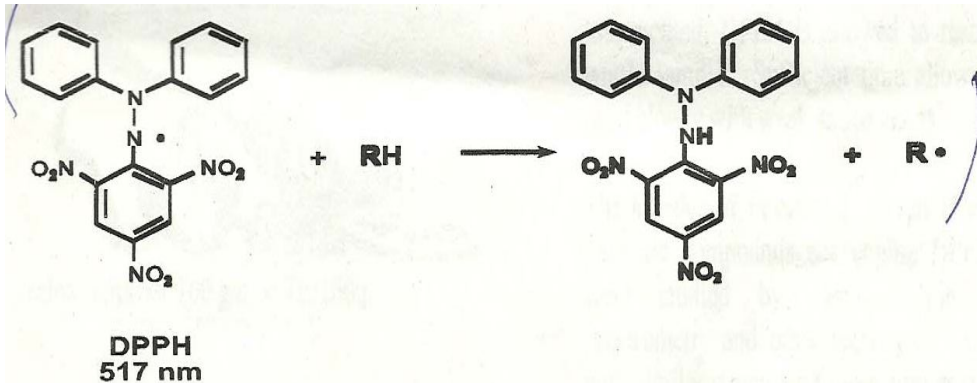

Fig: 1 The reaction of DPPH to converted in Free Radical

Plant Material:

Healthy and fresh Soybean seedwere selected and purchased from the local market at Jhansi : and also collected naturally.

Preparation of Samples:

Soybean seed was cleaned under running tap water excessive water was drained off. The soybean seeds were cut into small pieces and subjected to size reduction using kitchen blender with a kitchen mixer to get a thick paste, and kept at 20^{o}C for further analysis.

Preparation of Extract:
250 mg of plant sample was extracted with 10 ml of 100 % methanol and left it over night, Next day filtered with whatman filter paper and make up the volume up to 25 ml with 100% methanol.

Radical Scavenging Activity Using DPPH Method:
Radical Scavenging Activity (RSA) of the extracts was determined by DPPH method as described by **Blois[21](1958).** Different aliquots of 0.025, 0.05, 0.10, 0.25, 0.50 ml (equivalent to 25, 50, 100, 250 and 500 ppm of extract of soybean seed, the total volume was adjusted to 1.0ml with MeOH, 4ml of 0.1mM methanolic DPPH was added to these tubes and shaken well. The mixture was allowed to stand at room temperature for 20 min. the blank was prepared without extract (sample). The change in the absorbance of the sample was measured at 517 nm.
Radical scavenging activity was expressed as an inhibition percentage and was calculated using the formula.

$$RSA\% = \frac{\text{Blank OD-Sample OD}}{\text{Blank OD}} \times 100$$

Where, Blank OD = Optical Density of Blank solution
Sample OD = Optical Density of Sample solution

HPLC Analysis:
A water model 2690 HPLC equipped with a waters 2996 photodiode array detector (PDA) was used to separate, identify, and quantity isoflavones[169] separation of isoflavones was achieved using a 4 µm water Novapak C 18 stationary phase HPLC column (150mm x 3.9 I.D,.) with a Novapak C 18 stationary phase guard column and a 0.5 µm filter from Vydac. One millilitre of 100% methnol was vortexed and passed though a sample for injection. The mixture was vortexed and passed though a 0.2sµm syring filter prior to HPLC injection. The mobile phase consisted of 1% (V/V) acetic acid in water (solvent A) and 100% acetonitrile (solvent B) at a flow of rate of 0.6 ml/min. the sample injection volume was 10 µl , and component were eluted using the following solvent A was increased from 65 to 85% solvent A was re-equilibrated at 85% for 5 min. between each injection , a mixture of 85% solvent A and 15% solvent B was run for 20 min. the spectra were collected between **240 and 400nm** by PDA, and compound in the eluted were detected at **260nm.**

RESULT AND DISCUSSION

All results were obtained from a minimum of four independent experiment and the relevant means were calculated. Data were expressed on a dry weight basis.
The result of antioxidant analysis of four commonly consumed Soybean in March to June 2012.

(B) Physico Chemical Analysis

Table 1: Physico and Chemical properties

Parameter	Soybean
Acidity %	**0.20%**
pH	**6.70%**
Total soluble solids	**7.23%**
Ratio (solid/acidity)	**15.27%**
Tannin	**6.178%**

Some chemical properties of soybean are given in **Table-1** the acidity pH , total soluble solids, ratio (solid/acidity) and values of acidity, pH, total soluble solids, ratio (solid/acidity) and tannin were found. Values were 0.20%, 6.70, 7.23 g 15.27 And 6.178%.

(B) Total Phenolic:

The results of phenolic analysis of Soybeans are given in **Table2-3 .**

1.Prussian – Blue method:

The result of $FeCl_3/K_3[Fe(CN)_6]$ (Prussian Blue) system provides a sensitive method for quantitative determination of dilute concentration of polyphenolics in Soybeans solution. **Table 2** show determination[8, 22,23, 24, 25] the factor value in Tannic acid was standardized by using Prussian blue method.[26] The sensitivity of the test towards flavonoid compound is sufficient to determine concentration less than 10^{-4} M.

Table shows the phenolics compound present is 173.63% soybean. By this methods Spinach showed the highest polyphenolic content as compared to the other three Soybeans.

This test is based on the reduction by tannin and other polyphenols of ferric ion to ferrous ion, followed by formation of an Frricyanide ferrous ion complex. The coloured product (commonly known as Prussian blue) absorbs maximum at **725nm**. Initial the solution is fellow, the colour of the reagent. Increasing amount of Tannin result in the production of increasing amount of the blue pigment, which absorbs the red end of the spectrum.

Table: 2. Calculation of Percentage Total Phenolic in selected Soybean:

Seed	**OD**	$\frac{\text{OD x factor x 25 x 100}}{\text{Conc. x wt. of sample(mg)}}$	**Total Phenolic (%)**
Soybean	**0.587**	$\frac{\text{0.587x 29.58.2 x 25 x 100}}{\text{1 x 250}}$	**173.63**
Factor value = 29.58			

Solution however, appears green because the blue end of the spectrum is still masked by unrelated furrieyanide of the initial Ferricyanide concentration is sufficiently low, it will become noticeably depleted with higher amount of Tannin. The result is a deepening of the green colour followed by a change to blue colour.

2.Butanol – HCl Method:

Table :3. Calculation of Percentage Total Tannin in selected Soybean:

seed	**OD**	**%Dry Matter (Total Solid)**	**OD x 78.26 x Dillution factor / (% Dry Matter)**	**% Tannin contain**
Soybean	0.102	64.6	$\frac{0.102 \times 78.26 \times 50}{64.6}$	6.178
Factor = 78.26 **Dilution Factor (DF): Ratio of Final Volume/aliquot volume** **(final volume = aliquat +diluent)** **When: DF = 25/.5=50**				

The prescribed method for the Butanol – HCl Method is based on the amount of Total Tannin. The several Soybean had different Tannin Content in **Table 3**showed. The result of the present work to find percentage of Tannin soybean had 6.178%.

Vanillin – HCl Method:

Table: 4. Calculation of Percentage % Condensed Tannin in Selected Soybean see

Seed	**Absorbance**	**OD x factor x 25 x 100 / Conc. x wt. of sample(mg)**	**(%) C T**
soybean	**0.090**	$\frac{0.090 \times 2.6 \times 25 \times 100}{1 \times 250}$	**2.33**
Factor value = 2.6			

The % C Tannin of Soybeans is shown in **Table 4.** The Condensed Tannin of soybean was 2.33%.

Antioxidant Activity:

Free Radical Scavenging Activity by DPPH Method:

Free radical scavenging potential of Soybean extract at different concentration were tested by DPPH methods [8,22,23,24,25] and the results are presented in **Table-5** and **figure 2** It can be seen that the different Soybeans exhibition varying degrees of In *Vitro* total antioxidant capacity [19,20]. The average antioxidant of soybean 41.97% RSA with methanolic DPPH solution respectively.

Table 5. Calculation of Optical Density and % RSA of the selected Soybeans: Antioxidant activity

Different Concentration of DPPH solution in ppm	**Optical Density** Soybeans	**RSA% = Blank OD-Sample OD x100 / Blank OD** Soybeans
25	**0.103**	**40.11**
50	**0.111**	**35.46**
100	**0.116**	**32.55**
250	**0.097**	**43.60**
500	**0.072**	**58.13**
Blank OD = 0.172	**TOTAL DPPH VALUE = 41.97%**	

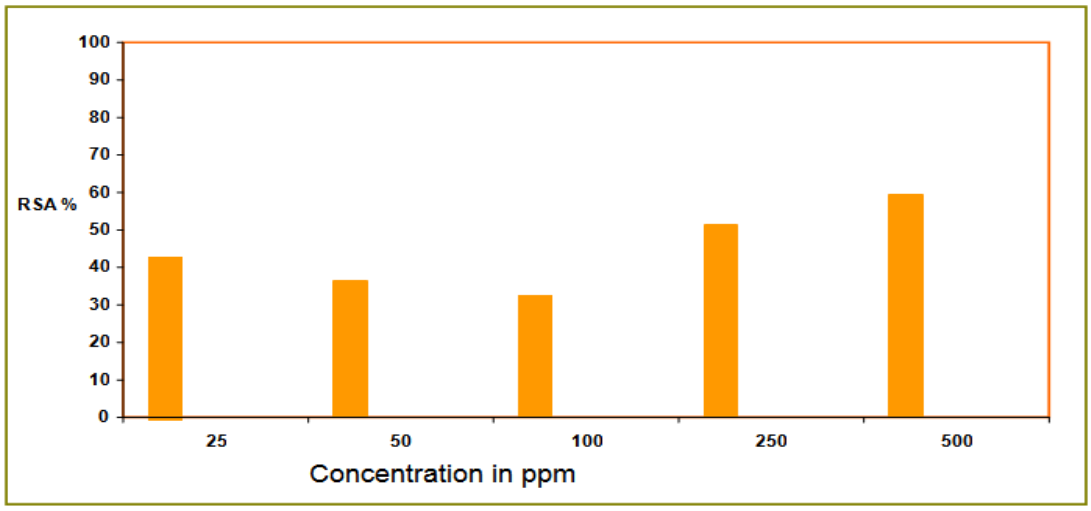

Fig. 2. Does response of radical savaging activity (RSA%) of Soybeans at different concentration by DPPH method.

In this method, the reducing power of various extract is directly reflected by the absorbance value AA of each Soybean extract is proportional to the increase in the absorbance of the reaction mixture. The essence of DPPH method is that the antioxidant react with DPPH (1, 1-diphenyl-2-picryl hydazyl) and convent it to 1, 1-diphenyl-2-picryl hydrazine with discoloration. The degree of discoloration at **517 nm** indicates the scavenging potential of the antioxidant as has been used as a measure of AA.

In general, MeOH extract of Soybeans showed higher AA as compared to other solvent extracts at comparative concentrations. It has been reported that the phenolic are the dominant antioxidants found in the natural products of plant sources **(prior and cao[10] 2000)** and the activity of various extracts is attributed to their hydrogen donating ability **(shimada et al[27] 1992)**. The free radicals cause anti – oxidation of unsaturated lipids in food **(kaur and perkins[28] 1991)** and antioxidants

interrupt the free radicals chain of oxidation and donate one hydrogen atom from the polyphenolic hydroxyl groups; there by forming stable and product which does not initiate or propagate further oxidation of lipid **(sherwing[29] 1978)**. The experimental data obtained indicate that the extracts are free radical inhibitors and primary antioxidants that react with free radicals.

HPLC Method

(Isoflavones analysis of soybeans)

A typical HPLC chromatography of Isoflavones in soybean is shown in figure Genistein, daidzein, and glycitein as well as their β-glucosides (acetylgenistin,acetydaidzein, acetylglycitin) 6-O-malonyl-β- glucosides (malonygenistin, malonydaidzein, malonyglycitin), internal standard were successfully separted and identified using the applied HPLC conditions. The average coefficient of variations for all 12 Isoflavones analysis each soybean cultivar was less than 5% (n=4).

There were significant differences in Isoflavones content thatincluded all aglycones and their glycoside conjugation as well as total daidzein, total genistein total glycitein, and total Isoflavones ($P<0.05$). The highest total Isoflavones content was 11.75 µmol/g soy in soybean , and lowest was 4.20 µmol/g in soybean, while the average of total Isoflavones in the soybean was 7.12 µmol/g soy. Among the Isoflavones , malonyl genistein content was the highest, followed by malonyl daizein and genistein, and glycitein was the lowest , total genistein content was the highest , followed by total daidzein and total glycitein, which was 64.7,32.9 and 2.4%of total isoflavone, respectively.

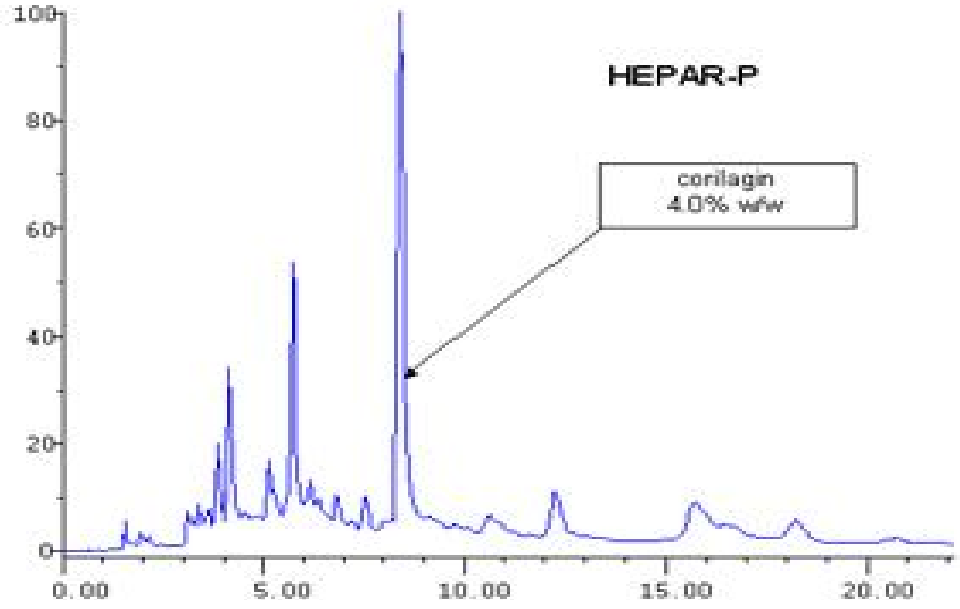

Figure 3. showing HPLC Chromatogram of isoflavones in Soybean

CONCLUSION

The advantage of the determination of antioxidant activity and total phenolic content in Soybean are:

- The value of the polyphenolic content is high.
- The results are a direct measure of soluble polyphenolic content.
- The test is so sensitive that no interfering colour is present at the dilutions used.
- A simple and rapid method for evaluating antioxidant activity has been developed. The antioxidant activity against DPPH methods are of the useful in characterizing the properties antioxidant activity of the substances found in the Soybeans.
- The antioxidant activity of Soybeans various considerable according to the type of Soybeans.
- The rapidity and simplicity of the test makes it ideal for use at the Soybean elevators.
- The health benefits from Soybeans sources and additional information of dietary intake is provided.

The formation of the Prussian blue complex offers a sensitive, versatile method for spectrophotometric determination of total polyphenols. The Main disadvantage of this or any other redox method is that is that no distinctions are made between tannins and other phenols. A measure of actual tannins present in the fruit is more accurately measured by the aqueous Prussian blue test when corrected by the salt extraction method or by the Vanillin test as corrected in this work.
Even these modifications cannot be said to definitely measure only tannins. Monomeric proanthocyanidins will give a positive Vanillin test and it is not known what effect polymerization will have on the extent of the reaction and the extinction coefficient of the product. The Prussian blue method always yields a complex with the same extinction coefficient, but some error is introduced when polyphenolics with varying hydroxylation patterns, degrees of polymerization, etc., are mixed in unknown proportions.

It must be remembered in all methods used that only polyphenolics extractable under the given conditions are being measured. Changing solvents after exhaustive extraction with another solvent has been known to bring out additional tannin **(Hillis and Swain, 1959)**.[30]

Antioxidants protect us because they can scavenge ROS before they cause damage to the various biological molecules, or prevent oxidative damage from spreading, e.g. by interrupting the radical chain reaction of lipid peroxidaton. The antioxidant defense systems in the human body are extensive and consist of multiple layers, which protect at different sites and against different types of ROS.

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