Antioxidant potential of aqueous extract of some food grain powder in meat model system

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Abstract In-vitro antioxidant activity of some food grains [*sprouted mung bean (Vigna radiata)*, *mung bean*, *sprouted chana (Cicer arietinum)*, *chana*, *corn (Zea mays)*, *methi (Trigonella foenum-graecum)* and *rajma (Phaseolus vulgaris)*] powder extracts (FGE) was estimated by DPPH free radical scavenging activity (SA) method. Total phenolics and reducing power were also estimated in these extracts. The antioxidant potential of these extracts was also estimated in a meat model system. Total phenolics in FGE ranged from 66.9 ± 3.4 to 248.6 ± 11.1 mg TAE/gdw respectively which differed significantly ($P < 0.01$) among various groups. These extracts also showed remarkable DPPH radical scavenging activity (43.9 ± 1.1 % to 69.9 ± 2.9 %). The reducing power_{OD700} was observed maximum ($P < 0.01$) in sprouted mung bean extract as compared to corn powder extract. The correlation between DPPH free radical SA v/s total phenolics was significant ($P < 0.01$) with $R = 0.775$. correlation between DPPH free radical SA v/s reducing power and total phenolics v/s reducing power was also significant ($P < 0.01$) with R value 0.907 and 0.682 respectively. FGE treated raw chicken breast meat showed better ($P < 0.01$) oxidative stability than control groups.

Keywords Food grains · DPPH · Reducing power · Total phenolics · Meat lipid · TBARS

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Introduction

Interest in natural antioxidants for improved food stabilisation has intensified over the past years. For the food industry, moving to natural antioxidants is a potential goldmine that offers a “green” label for food stabilisers.

Determining antioxidant capacity in vitro and/or in some food model system has thus become a very active research topic, and an alphabet soup of assays has evolved to screen natural materials and identify likely candidates that will extend the shelf life and quality of foods.

Plant-based food items contain a wealth of phytochemicals, and many have antioxidant properties (Halliwell et al. 1995; Hollman and Katan 1997; Duthie et al. 2000; Yogesh and Ali 2012). Grains, fruits and vegetables have been considered as excellent sources of antioxidants. Vitamins C and E, Phenolic compounds and carotenoids have been thought to be responsible for most of the antioxidant activity in foods (Esterbauer et al. 1991; Jialal et al. 1990; Duthie and Crozier 2000). Many workers recently reported antioxidative effect of aqueous extract of some plant and fruit byproducts in meat and meat products (Yogesh and Ali 2012; Naveena et al. 2008).

Thus the main objective of this study is to investigate the In-vitro antioxidant activity of some food grain [*sprouted mung bean (Vigna radiata)*, *mung bean*, *sprouted chana (Cicer arietinum)*, *chana*, *corn (Zea mays)*, *methi (Trigonella foenum-graecum)* and *rajma (Phaseolus vulgaris)*] powders extracts by DPPH free radical scavenging activity (SA) method. Total phenolics and reducing power were also estimated in these extracts. The antioxidant potential of these extracts was also estimated in a meat model system at refrigeration temperature.

Materials & methods

Preparation of aqueous solution of different powders

Different ingredients like different grains [*mung bean* (*Vigna radiata*), *chana* (*Cicer arietinum*), *corn* (*Zea mays*), *methi* (*Trigonella foenum-graecum*) and *rajma* (*Phaseolus vulgaris*)] and boneless chicken breast meat (6 wks of age, 2 h post slaughter) obtained from local market. To sprout the beans, bean were soaked overnight (37 °C) and then allowed to sprout for 48 h (37 °C), after that they were dried in tray driers at 45 °C. Powder was made in heavy duty kitchen grinder. These grinded powders then sieved through 0.6 mm mesh size sieve. About 100 g of different dried powders were mixed with 1 l warm sterilized distilled water (45 °C) and left for 2 h. The extracts obtained after filtration with grade A filter (S.D. fine chem. Ltd.) were used to evaluate different parameters.

Treatment of chicken breast meat with extracts

Chicken breast meat was minced through mincer (5 mm plate). One Kg minced meat grinded with 20 g salt, 50 ml ice cold extract [sprouted mung powder (SMP), mung powder (MP), sprouted chana powder (SCP), chana powder (CP), corn powder (CoP), methi powder (MeP), rajma powder (RaP)] and 50 ml vegetable oil for 5 min in a kitchen grinder, in control samples 50 ml extract was replaced with 50 ml distilled water keeping other contents same as that of treated samples. These all treated (SMP, MP, SCP, CP, CoP, MeP, RaP) and control ground meat (GM) were then filled in LDPE and stored at 4 °C for further studies.

Total phenolics

The concentration of total phenolics in extract was determined by the Folin-Ciocalteus (F-C) assay (Escarpa and Gonzalez 2001) with slight modifications. Suitable aliquots of extracts were taken in a test tube and the volume was made to 0.5 ml with distilled water followed by the addition of 0.25 ml F-C (1 N) reagent and 1.25 ml sodium carbonate solution (20 %). The tubes were vortexed and the absorbance recorded at 725 nm after 40 min. The values were reported as mg of tannic acid equivalent (TAE) by reference to tannic acid standard curve and the results were expressed as milligrams of TAE per gram dry weight (gdw) of powder.

Measurement of reducing power

The reducing power was quantified by the method described by Jayaprakasha et al. (2001). The 2.5 ml extract was mixed with 2.5 ml phosphate buffer (200 µM, pH 6.6) and incubated with 2.5 ml potassium ferricyanide (1 %w/v) at 50 °C

for 20 min. At the end of incubation, 2.5 ml of 10 % trichloroacetic acid solution was added and centrifuged at 9,700 g for 10 min. The supernatant was mixed with 5 ml distilled water and 1 ml ferric chloride (0.1 %w/v) solution. The absorbance was measured at 700 nm. Increase in absorbance of the reaction indicated the reducing power of the extract.

DPPH radical scavenging activity

The method of Singh et al. (2002) was employed to assess the ability of extracts to scavenge 1, 1-diphenyl 1-2- picrylhydrazyl (DPPH) radicals; with slight modification. The 400 µl extract diluted with 1,600 µl, 0.1 M Tris-HCl buffer (pH 7.4) was mixed with 2 ml of DPPH (500 µM) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min and the absorbance was measured at 517 nm, the scavenging activity (SA) was calculated by the following equation:

Scavenging activity(SA)%

$$= [1 - (\text{Absorbance sample}/\text{Absorbance Blank})] \times 100$$

Estimation of thiobarbituric acid reactive substances (TBARS)

The thiobarbituric acid reactive substances (TBARS) were determined at 0 h and after 24 h by using the extraction method described by Witte et al. (1970) with slight modification. Four gm sample was homogenized with 20 % trichloro acetic acid solution (20 ml) and the slurry was centrifuged at 3,000 g (MP 400R Eltek Ltd., India) for 10 min. 2 ml of supernatant was mixed with equal volume of freshly prepared 0.1 % thiobarbituric acid in glass test tubes and heated in water bath 100°C for 30 min followed by cooling under tap water. The absorbance of the mixture was measured at 532 nm using UV-VIS spectrophotometer (UV-1800 PharmaSpec, SHIMADZU, Japan) and the TBARS values were calculated using a TBA standard curve and expressed in mg malonaldehyde/kg.

Statistical analysis

Data are presented as mean $\pm$ SD of three replicate experiments each with three measurements ($n=9$). One-way analysis of variance (ANOVA) followed by Duncan's test were performed to determine statistical differences between groups with the aid of SPSS software version 11.0 (SPSS, Chicago, IL, USA). Statistical significance was defined as $P<0.05$ for all tests. Correlation values (R) were calculated for DPPH free radical SA, total phenolics and reducing power in all combinations. Correlations were considered significant at the level $P<0.05$.

Results and discussion

Total phenolics

Phenolic compounds are believed to account for a major portion of the antioxidant capacity in many plants (Duthie and Crozier 2000). Thus it is important to know the phenolic contents and their efficacy as natural antioxidant. Extractability of phenolic compounds is governed by the type of solvent (polarity) used, degree of polymerization of phenolics, interaction of phenolics with other food constituents, as well as the extraction time and temperature. Thus, there is no uniform or completely satisfactory procedure that is suitable for extraction of all phenolics from plant materials (Naczka and Shahidi 2004). In present investigation total phenolics contents in aqueous extract were differed significantly ($P<0.01$) between different groups (Fig. 1). Highest contents (248.6 ± 11.1 $\mu\text{g TAE/gdw}$) were found in MP group and lowest contents were observed for CoP group. The contents ranged (from 248.6 ± 11.1 to 66.9 ± 3.4 $\mu\text{g TAE/gdw}$) as $\text{MP}>\text{CP}\geq\text{SMP}>\text{MeP}\geq\text{RaP}>\text{SCP}>\text{CoP}$. Interestingly total phenolics were found less in sprouts than whole grain for both mung beans and chana beans. This suggests conversion of water soluble phenolics to other compounds during germination. Other workers also reported phenolics contents in legumes and condiments. Xu and Chang (2008) have reported phenolic content of green pea, yellow pea, and chickpea as 1.22, 1.38, and 1.44 mg GAE/g, respectively. Cowpea (red and brown), soybean, common bean (black, red, brown, and beige), peanut, fenugreek and horse gram showed high phenolic content (2.0–6.4 mg GAE/g) (Marathe et al. 2011).

Reducing power

The overall ability of a substance to reduce other compound of interest is determined by reducing power assay. In this assay absorbance of produced chromogen is measured at 700 nm. Increase in absorbance of the reaction indicated increase in the reducing power of the sample. In present investigation the reducing power differed significantly ($P<0.01$) (Fig. 1) between different groups. Reducing power was highest in SMP followed by other groups. Here the reducing power of germinated beans was observed more than ungerminated beans.

DPPH radical scavenging activity

DPPH assay is routinely practiced for assessment of free radical scavenging potential of an antioxidant molecule and considered as one of the standard and easy colorimetric methods for the evaluation of antioxidant properties of pure compounds (Mishra et al. 2011). Though this radical has limited similarities with peroxy radicals, this assay is in

Fig. 1 Total phenolic contents ($\mu\text{g TAE/gdw}$), Reducing power (OD₇₀₀) and DPPH free radical SA (%) of different food grain powder. Bar showing different labels differed significantly ($P<0.01$) ($n=3$)

commonly used to measure the antioxidant content of wheat grain and bran, vegetables, conjugated linoleic acids, herbs, edible seed oils, and flours in different solvent systems including ethanol, aqueous acetone, methanol, aqueous alcohol, and benzene (Cheng et al. 2006). DPPH is a stable radical in solution and appears purple colour absorbing at 517 nm wavelength. This assay is based on the principle that DPPH on accepting a hydrogen (H) atom from the scavenger molecule i.e. antioxidant, resulting into reduction of DPPH free radical to DPPH stable compound (Fig. 2); the purple colour changes to yellow with concomitant decrease in absorbance at 517 nm. The colour change is monitored spectrophotometrically and utilized for the determination of parameters for antioxidant properties.

Fig. 2 DPPH free radical reaction with antioxidant agent (AH; free radical scavenger) and formation of stable form. $A^\bullet$ scavenger free radical is very stable as compared to other radicals

In present work DPPH free radical SA was differed significantly ($P < 0.01$) between groups (Fig. 1). Maximum SA was noted in SMP (68.8 ± 1.0 %), MP (69.8 ± 0.4 %), MeP (69.9 ± 2.9 %) and RaP (67.0 ± 0.1 %) groups. In these groups SA were found to be nonsignificant between each

other. Results showed that there was no significance observed for SA between ungerminated and germinated grains. Least SA was observed in CoP (43.9 ± 1.1 %) group.

Correlation between DPPH free radical SA, reducing power and total phenolics

Fig. 3 Correlation of DPPH free radical SA (antioxidant activity), reducing power and phenolic contents of different food grain powders. **a** The plot of DPPH free radical SA v/s reducing power; **b** total phenolic content versus DPPH free radical SA; **c** total phenolic content v/s reducing power

The correlation between DPPH free radical SA v/s total phenolics, DPPH free radical SA v/s reducing power and total phenolics v/s reducing power are depicted in Fig. 3a–c. The correlation between DPPH free radical SA v/s total phenolics was significant ($P < 0.01$) with $R = 0.775$. Correlation between DPPH free radical SA v/s reducing power and total phenolics v/s reducing power was also significant ($P < 0.01$) with R value 0.907 and 0.682 respectively. Phenolic compounds have been reported to have multiple biological effects, including antioxidant activity (Ahn et al. 2007). One of the major groups of compounds present in samples is polyphenols such as flavonoids that may act as scavenging free radicals or prevent their formation. This fact may contribute to the ability of phenolics to prevent oxidation. Barclay et al. (1997) studied the antioxidant properties of phenolic

Table 1 TBARs (mg Malonaldehyde/kg) values of treated and control raw chicken ground meat during refrigerated (4 ± 1 °C) storage

Group	TBARs mg malonaldehyde/Kg	
	At 0 h.	After 24 h.
SMP	0.52 ± 0.10	0.92 ± 0.06^b
MP	0.51 ± 0.01	0.62 ± 0.07^c
SCP	0.52 ± 0.10	0.54 ± 0.08^{cd}
CP	0.47 ± 0.11	0.43 ± 0.06^d
CoP	0.52 ± 0.05	0.44 ± 0.03^{cd}
MeP	0.48 ± 0.02	0.58 ± 0.06^d
RaP	0.48 ± 0.10	0.59 ± 0.12^d
Control	0.49 ± 0.11	1.18 ± 0.06^a

Column values showing different superscripts differed significantly ($P < 0.01$) ($n = 3$)

lignin model compounds (among them 4-propylguaiacol, eugenol, isoeugenol and 4-allyl-2,6-dimethoxyphenol), reporting a higher antioxidant capacity of these compounds with respect to the commercial 2, 6-di-tert-butyl-4-methylphenol (BHT). The antioxidant activities of eugenol, guaiacol and 4-allyl-2, 6-dimethoxyphenol were also reported by Ogata et al. (1997). The results from present investigation also suggests the positive correlation between total phenolics, reducing power and free radical SA i.e. antioxidant potential.

Oxidative stability of meat lipids

The TBARS values correlates with oxidative stability of meat products and usually presented as mg malonaldehyde per Kg of product. This value determines the extent of lipid oxidation. The antioxidant effect of the treatments, measured as the change in lipid oxidation value during 24 h of storage, is shown in Table 1. The initial concentrations of TBARS in the control and all treated meat samples were between 0.47 ± 0.11 and 0.52 ± 0.10 mg malonaldehyde per Kg and not significantly different. Treated groups showed significant ($P < 0.01$) less amount of TBARS as compared to control groups after 24 h of storage. Minimum values were recorded in CP group. Results also showed more oxidative stability in non germinated (non sprouted) beans powder extract treated meat samples than sprouted beans powder extract treated meat samples. Results obtained from present study indicating that these extracts effectively protected against lipid oxidation of raw chicken meat.

The phenolic compounds of these extracts might be involved in the inhibition of lipid oxidation, because phenolic compounds can inhibit free radical formation and the propagation of free radical reactions through the chelation of transition metal ions, particularly those of iron and copper (Brown et al. 1998). Lee and Ahn (2003) found that phenolic compounds such as gallate and sesamol were effective antioxidants in reducing TBARS in turkey breast during 5 days of storage. Chen et al. (1999) reported that phenolic compounds such as quercetin were also effective in preventing lipid oxidation in both raw and cooked turkey during 7 days of storage. In another study methanolic extract of some legume were effective in the protection of lipids and non-lipids against various oxidative model systems.

Conclusion

In conclusion, the present results showed that different food grains extract showed remarkable antioxidant activity. Reducing power was also higher in these extracts. These

activities showed very good correlation with total phenolics found in these food grain extract. These extracts treated raw meat also show better oxidative stability of lipid. This might have some commercial application in food processing. The mechanism of the inhibitory effects by which these extract protects against oxidation may involve reactive oxygen scavenging and metal binding.

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