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Studies on walnut green husk powder properties for utilization in processed food products

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Abstract
The study was conducted to evaluate the composition of walnut green husk powder (WGHP) along with its antioxidant and antimicrobial activities to explore the possibilities for utilization in processed and functional food products. The clean walnut green husk was dried in hot air oven drier at 48±2 °C for 36 hrs and ground to fine powder in an electric mixer. The powder was analyzed for total fiber, total phenolics, antioxidant (DPPH and ABTS radicals scavenging) and antimicrobial (TPC, Yeas & mold counts) activities. Results revealed that walnut green husk powder was very rich source of total phenolics (46.60 mg/g Gallic acid Equivalent) and crude fiber content (48.42%). It showed very good antioxidant (DPPH value 85%, and ABTH value 59%) properties, which were almost like the values of BHT at 0.15% level. The samples treated with 0.15% WGHP showed significantly higher reduction values as natural antimicrobial agent for TPC (23%) and Y&MC (39%). It is concluded that walnut green husk powder can be used as natural antioxidant and antimicrobial agent for development of functional food products.

Keywords: Walnut green husk, composition, antioxidant, antibacterial

Introduction
The processing of food products leads to pose a health hazard due to high amount to added salt and fat that somehow is proved to be a predisposing factor for cardiovascular diseases, diabetes mellitus and cancer [1]. Following microbial deterioration, lipid oxidation is a primary mechanism and the main cause of loss in quality of processed products. The changes in quality are manifested by adverse changes in flavor, color, texture and nutritive value, and the possible production of toxic compounds.
However, synthetic antioxidants have been successfully used to prevent oxidation in fresh and processed foods, but these are suspected to be carcinogens. This is one of the reasons for the increased demand of the healthy (natural and functional) foods [2]. Different types of plants like herbs, fruits and vegetables possess bioactive compounds that exhibit antioxidant and antimicrobial properties. Numerous types of natural antioxidants with various activities have been identified but a lot of attention has recently been drawn to the addition of polyphenols to foods and biological systems, due to their known abilities to scavenge free radicals [3].
The outer green thick layer of the walnut fruit is termed its walnut green husk, which is an abundant agricultural waste crop produced upon the harvesting of fruit and its processing. If this waste material is not adequately disposed of, it can cause environmental pollution [4], but recently, the walnut husk has received an interest in modern pharmacology and has been extensively used in traditional medicine for the treatment of skin diseases and the alleviation of the pain [5] due to its excellent antioxidant activities. Hence, the compositional and phytochemical studies of this husk as a source of crude fiber and natural compounds with antioxidant and antimicrobial properties may enhance its use to improve the functional properties and shelf life of processed products.

Materials and methods
As researcher (MVSc student) belongs to Afghanistan, he procured Walnut (Juglans regia L.) green husk from his own farm and processed it in department of L.P.T, COVS, LUVAS, Hisar for further use. Walnut green husk was washed thoroughly under running tap water and wiped out completely with muslin cloth. Husks were peeled off with the aid of a stainless-steel peeler and peel free from pulp was collected. The clean husks were dried in hot air oven drier at 48±2 °C for 36 hrs and ground to fine powder in an electric mixer.

Crude fibre estimation was done in Fibra plus apparatus, Pelican Equipments, Chennai. Fat Fat free dried sample (1 g) was weighed in a crucible and 150 ml 1.25% hot H_2SO_4 was added. It was heated to boiling for 45 min under condensers. Acid was drained by vacuum suction and sample was washed with distilled water. Precipitates were again boiled for 45 min with hot 150 ml of 1.25% NaOH. Alkali was drained by vacuum suction and the sample was washed by distilled water. It was dried at 100 °C to constant weight. Ashing was carried out in muffle furnace at 550±5 °C for 2 h. Crucibles were cooled in desiccator and weighed.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of insoluble matter - Weight of ash}}{\text{Weight of sample}} \times 100$$

The polyphenol content was quantified by Folin-Ciocalteau's reagent and was expressed as Gallic acid equivalents [6]. For preparation of sample extract, same procedure as mentioned was followed for DPPH [7] and. ABTS [8] radicals scavenging activity. Antimicrobial (TPC, Yeas & mold counts) activities were measured as per APHA [9] method and moisture, protein, fat, and ash content of the product was estimated using AOAC [10] methods.

Statistical analysis

Data was analyzed statistically on 'SPSS-16.0' (SPSS Inc., Chicago, II USA) software package as per standard methods of Snedecor and Cochran [11]. Duplicate samples were drawn for each parameter and the whole set of experiment was repeated three times. The average values were reported along with standard error. The statistical significance was estimated at 5% level ($p<0.05$) and evaluated with Duncan's Multiple Range Test (DMRT).

Results and Discussion

Composition

The composition of walnut green husk was evaluated for moisture, protein, fat, ash, total phenolics and crude fiber (Table 1) on dry and wet bases. It was found that walnut green husk powder was a rich source of phenolics compounds (46.60 mg/g, Gallic acid Equivalent) and crude fiber (48.42%,). These results are within the range as reported by Oliveira *et al.* [8] and Cosmulescu *et al.* [12].

Table 1: Composition of walnut green husk. (Mean ±S.E. n=6)

Parameters	(%) Wet basis	(%) Dry mater basis
Moisture	88.21±3.33	5.31±0.23
Protein	2.05±0.11	18.72±1.16
Fat	1.01±0.09	7.91±0.61
Ash	2.40±1.04	19.63±1.23
Crude fiber	6.32±0.31	48.42±2.13
Total Phenolics (Gallic acid Equivalent mg/g)	Note done.	46.60±1.74

DPPH inhibition (%)

The samples prepared with BHT at 0.02% (highest recommended level) and WGHP at 0.1, 0.15, 0.2, 0.25, 0.3 and 0.4 per cent levels were compared for DPPH radical scavenging activity (Fig. 1). Percent inhibition DPPH radical scavenging activity of WGHP (0.15% level) was almost similar to the value of BHT. Further addition of WGHP after 0.2% level did not significantly increase the% DPPH inhibition activity. It showed strong antioxidant property as natural antioxidants at 0.15g/100g level.

The antioxidant activity of phenolic compounds in plants is mainly due to their redox properties and chemical structure, which can play important roles in neutralizing free radicals, chelating transitional metals, and quenching singlet and triplet oxygen molecules through delocalizing or decomposing peroxides. These properties are linked to beneficial health functionality of phenolic antioxidants due to their inhibitory effects against development of many oxidative-stress related diseases [13, 14].

Stampar *et al.* [15] identified thirteen phenolic compounds in walnut husks: chlorogenic acid, caffeic acid, ferulic acid, sinapic acid, gallic acid, ellagic acid, protocatechuic acid, syringic acid, vanillic acid, catechin, epicatechin, myricetin, and juglone and reported their potential use as antioxidant.

ABTS inhibition {%)

ABTS assay is based on the ability of compounds to react with ABTS free radical cation generated in the assay system. ABTS decolourization assay is an excellent tool for determining antioxidant activity of hydrogen denoting antioxidants.

ABTS radical scavenging activity (% inhibition) was almost similar to the value of BHT (0.02%) level and in WGHP (0.15%) level and it showed strong antioxidant property as natural antioxidant at 0.15g/100g level (Fig 2). Further addition of WGHP after 0.2% level did not significantly increase the ABTS inhibition activity. The high scavenging activity of WGHP might be due to their respective high phenolics contents. Gorinstein *et al.* [16] reported linear correlation between phenolics content and antioxidant potential of compounds.

Antimicrobial activity of WGHP

Antimicrobial activity of Walnut green husk powder has been presented in Fig. 3. The samples treated with 0.15% WGHP showed significantly higher reduction values as natural antimicrobial agent for TPC and Y&MC as compared to control reduction values. Further addition of WGHP after 0.15% did not affect significantly the antimicrobial activity and it showed very good result as natural antibacterial agent at 0.15% level. The study of antimicrobial capacity of plant phenolics is well known [17, 18] and our results are in accordance with the findings of Oliveira *et al.* [5], who screened the walnut green husks aqueous extract for their antimicrobial properties against B. *cereus*, B.*subtilis*, S. *aureus* and E. *coli*, and reported that all the tested extracts revealed antimicrobial activity showing different selectivity and minimum inhibitory concentrations for each microorganism.

Walnut green husk powder is a rich source total phenolics and crude fiber content. The powder showed very good antioxidant and antimicrobial properties. The DPPH value (85%) and ABTH value (59%) were almost like the values of BHT at 0.15% level. The samples treated with 0.15% WGHP showed significantly higher reduction values as natural antimicrobial agent for TPC (23%) and Y&MC (39%). It is concluded that walnut green husk powder can be used as natural antioxidant and antimicrobial agent for development of functional processed food products.

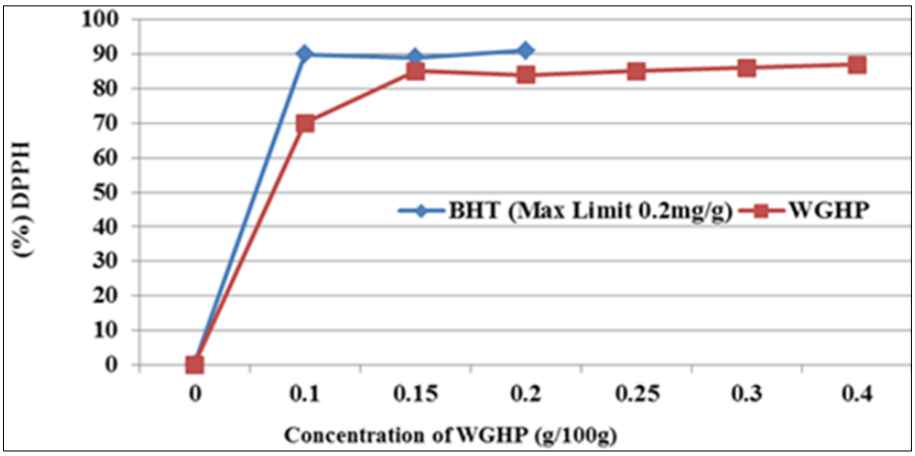

Fig 1: Scavenging effect on DPPH of walnut green husks powder (WGHP) at different concentrations. (Mean±S.D, n=6)

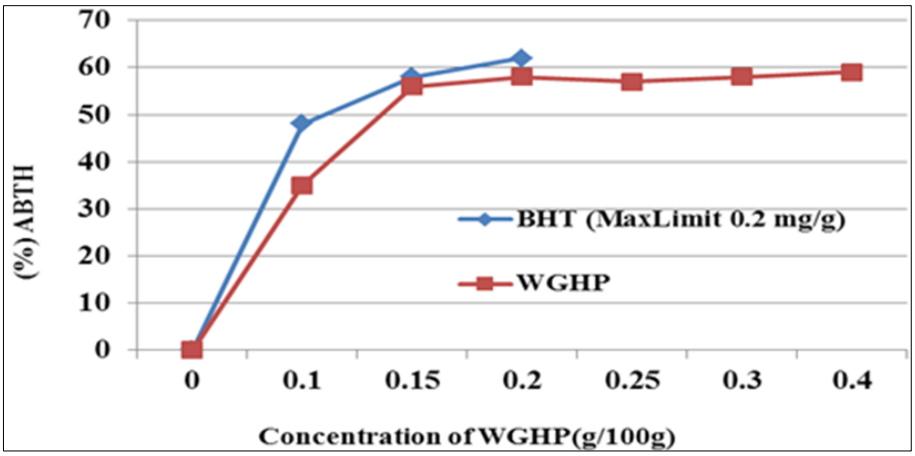

Fig 2: Scavenging effect on ABTS of walnut green husks powder (WGHP) at different concentration. (Mean ±S.D, n=6)

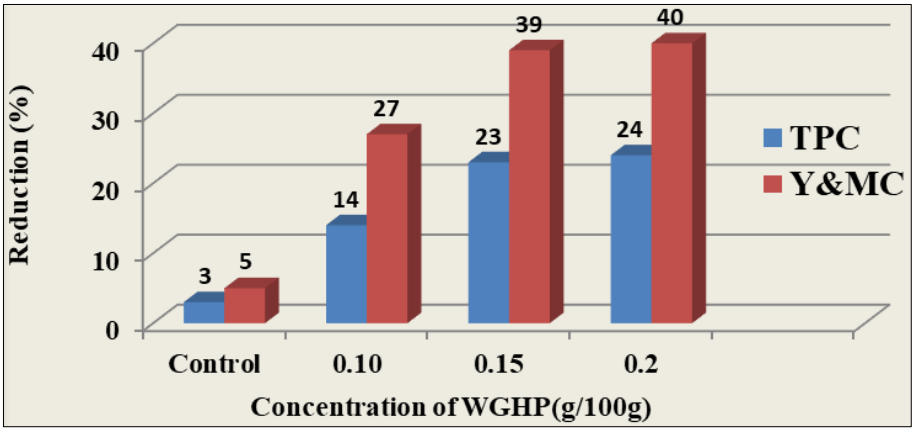

Fig 3: Antimicrobial activity of Walnut green husk powder

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