

COMPARATIVE STUDY ON ANTIOXIDANT PROFILE OF DIFFERENT PEELS BY USING DIFFERENT EXTRACTION SOLVENTS

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ABSTRACT

Growing interest in the replacement of synthetic food antioxidants by natural ones has fostered research on vegetable sources and screening of raw materials to identify new antioxidants. The food-processing industry generates substantial quantities of phenolic-rich by-products that could be valuable natural sources of antioxidants. The objective of this study was to investigate how two commonly used solvents affected the yields of phenolics, flavonoid and the antioxidant properties of extract from eggplant, beetroot and potato peel. Among the three extracts eggplant exhibited a high percentage of phenolic content of 64.3 mg/g, total flavonoid content of 0.8 mg/g and antioxidant activity of 75.6% in potato peel when extracted with ethanol as solvent in comparison to beetroot and eggplant peel extract. Several antioxidant related phytochemical composition namely Total Phenolic Content (TPC), Total Flavonoid Content (TFC) were investigated. In addition antioxidant activities were tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging. The result showed that ethanol as a solvent exhibit the highest antioxidant profile among three peels. Since different antioxidant compounds have different mechanisms of action. On the basis of the results obtained, eggplant, beetroot, potato peel extracts could serve as natural antioxidants owing to their significant antioxidant activity. Therefore they could be used as preservative ingredients in the food and/or pharmaceutical industries

Keywords: Different peels, Extraction solvents, Antioxidant, TPC, TFC, Antioxidant activity.

INTRODUCTION

Antioxidants occur naturally in many foods and are essential for our health. They include Vitamin C found in fruit and vegetables and vitamin E found in seeds and nuts. Antioxidants, both natural and synthetic, are used by the food industry as food additives to help prolong the shelf life and appearance of many foodstuffs. Foods that contain vegetable or animal fat go rancid when exposed to oxygen, heat, moisture or the action of enzymes. The speed at which this

takes place depends on a number of factors including the source of the oil or fat and how it is stored. Most vegetable oils contain naturally occurring antioxidants such as vitamin E.

The majority of antioxidants used in this way are synthetically manufactured.

Vegetable and fruits processing in India generates substantial quantities of waste. It had been previously reported that these wastes and by-products of fruits are an abundant source of antioxidant polyphenols (**Balasundaram et al., 2006**). These peels and pomace are a source of sugars, minerals and organic acids, dietary fibers and phenolics which have a wide range of actions which includes antioxidants, antimutagenic, cardio preventive, antibacterial and antiviral activities..

(**Shi and Maguer 2001**). It may be defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Antioxidants present in fruits and vegetables including ascorbic acid, carotenoids, flavonoids, hydrolysable tannins, are supposed to play an important role in the prevention of disease.

Eggplant is ranked as one of the top ten vegetables in terms of oxygen radical scavenging capacity due to the fruit's phenolic constituents. The eggplant is a vegetable typical of the Mediterranean diet is not only a good and versatile vegetable in the kitchen, but also a wealth of powerful antioxidants, for which it is a valuable ally in the prevention of major diseases, including cardiovascular disease, diabetes, tumors. It contains chlorogenic acid and Nasunin, two molecules that have significant activity against free radicals, that, as now widely confirmed, play a central role in phenomena such as aging, inflammation, cardiovascular disease, cancer. The Nasunin, in particular, is an anthocyanin, a purple pigment, belonging to the family of flavonoids, which together with other anthocyanins imparts to the eggplant its characteristic color.

The beetroot is the taproot portion of the beet plant, usually known in North America as the beet, also table beet, garden beet, red beet, or golden beet. It is one of several of the cultivated varieties of *Beta vulgaris* grown for their edible taproots and their leaves (called beet greens). These varieties have been classified as *B. vulgaris* subsp. *Vulgaris*, Other than as a food, beets have use as a food colouring and as a medicinal plant. Many beet products are made from other *Beta vulgaris* varieties, particularly sugar beet.

The potato is a starchy, tuberous crop from the perennial nightshade *Solanum tuberosum* L. Most of the wastes from potato plants arise from peeling, trimming, slicing, cleaning and rinsing operations, and the discharge of these liquid and solid wastes creates a pollution problem. Potato peel has been found to contain phenolic acids. However, convincing evidence for the free radical-scavenging activity of potato peel extract.

MATERIALS AND METHODS

Materials

Raw materials: Eggplant, Beetroot and Potato peels were obtained from the local market, Allahabad, India. The comparative study on antioxidant profile of different peels by using different extraction solvent, was carried out in Department of Food Process Engineering, Vaugh School of Agricultural Engineering and Technology, SHIATS.

Experimental Procedure:

Extraction of antioxidant from peels:

By using ethanol as a solvent

The dried powders of peels were extracted by cold percolation method using ethanol as a solvent, (Parekh and Chanda, 2007)

By using 80% aqueous methanol as a solvent

The dried powders of peels were extracted by using 80% aqueous methanol as a solvent, (Mazza and Gao, 1998)

Determination of Extraction yield:

The residues obtained after filtration were weighed to obtain the extraction yield.

Extraction yield (%) = (weight of the residue)/(total weight of the peel powder) × 100

Determination of Total Phenolic:

The total phenol content was determined according to Folin-Ciocalteu's reagent method (Mc Donald *et al.*, 2001). 0.5 ml of extract and 0.1 ml (0.5 N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml of 20% sodium carbonate solution was added and further incubated for 30 min. at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control. The phenolic content was expressed as gallic acid equivalents using the following linear equation based on the calibration curve: $y = 1.170x - 0.012$; $R^2 = 0.987$, where y is the absorbance and x is concentration as gallic acid equivalents (mg/g). Total phenol values are expressed in terms of gallic acid equivalent

Determination of Total Flavonoids:

The flavonoid content was determined according to aluminium chloride colorimetric method (Chang *et al.*, 2002). The reaction mixture consisting in a final volume of 3 ml, 1.0 ml of sample (1 mg/ml) 1.0ml methanol and 0.5 ml of (1.2%) aluminium chloride and 0.5 ml (120 mM) potassium acetate was incubated at room temperature for 30 min. The absorbance of all the samples was measured at 415 nm. Quercetin was used as positive control (Kanerla *et al.*, 2009). Flavonoid content is expressed in terms of Quercetin equivalent. Total flavonoid content were calculated as quercetin (mg/g) using the following equation based on the calibration curve: $y = 0.145x + 0.055$; $R^2 = 0.993$, where x was the absorbance and y was the quercetin equivalent (mg/g).

DPPH radical-scavenging activity:

The DPPH assay was utilised with some modifications. The stock reagent solution (1×10^{-3} mol L⁻¹) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at -20°C until use. The working solution (6×10^{-5} mol L⁻¹) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, as measured using a spectrophotometer. Extract and synthetic antioxidant (TBHQ, BHA and BHT in ethanol) solutions of different concentrations (0.1 mL of each) were vortexed for 30 s with 3.9 mL of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also analysed. Scavenging activity was calculated as follows:

$$\text{DPPH radical-scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A is the absorbance at 515 nm.

Table 1 Statistical analysis for comparative analysis of antioxidant profile using ethanol as solvent.

Peels	Total Phenolic Content (mg/g)	Total Flavonoid Content (mg/g)	Antioxidant Activity (%)
Eggplant Peel	64.3	0.8	75.6
Beetroot Peel	13.6	0.7	66.3
Potato Peel	14.5	0.5	86.3
F-Test	S	S	S
S.Ed (\pm)	0.308	0.006	0.760
C.D (P=0.05)	0.616	0.013	1.531

Table 2 Statistical analysis for comparative analysis of antioxidant profile using 80% aqueous methanol as solvent.

Peels	Total Phenolic Content (mg/g)	Total Flavonoid Content (mg/g)	Antioxidant Activity (%)
Eggplant Peel	44.7	0.3	65.9
Beetroot Peel	11.9	0.2	50.4
Potato Peel	15.3	0.7	70.2
F-Test	S	S	S
S.Ed (\pm)	0.239	0.004	0.621
C.D (P=0.05)	0.479	0.008	1.243

Statistical Analysis

All experiment was determined 3 times and the results were reported as mean. The data recorded during the course of investigation were statistically analyzed by the 'Analysis of Variance- One Way Classification'.

RESULTS AND DISCUSSION**Proximate composition of materials**

Moisture, protein, fat, ash, were determined according to standard methods (AOAC, 2005) given in Ranganna, 1986 respectively.

Table 3 Statistical analysis for comparison in nutritional value of different peels.

Peels	Moisture Content (%)	Fat (%)	Ash (%)	Protein (%)
Eggplant Peel	8.49	1.15	4.42	0.52
Beetroot Peel	7.92	0.25	4.11	0.25
Potato Peel	6.84	0.15	4.05	0.05
F-Test	S	S	S	S
S.Ed (\pm)	0.077	0.005	0.041	0.0027
C.D (P=0.05)	0.161	0.010	0.083	0.0054

Total Phenolic Content & Total Flavonoid Content when extracted with ethanol/80% aqueous methanol as solvent:

TPC was found maximum in eggplant peel (64.3 mg/g), minimum in beetroot peel (13.6 mg/g) whereas potato peel showed the TPC of (14.5 mg/g). When extracted with ethanol as a solvent. TPC was found maximum in eggplant peel (44.7 mg/g), minimum in beetroot peel (11.9 mg/g) whereas potato peel showed the TPC of (15.3 mg/g). When extracted with 80% aqueous methanol as a solvent. (Somawathi and Rizliyal, 2014) TFC found in eggplant peel (0.8 mg/g), in beetroot peel (0.7 mg/g) whereas potato peel showed the TFC of (0.5 mg/g) when extracted with ethanol as solvent. TFC found in eggplant peel (0.3 mg/g), minimum in beetroot peel (0.2 mg/g) whereas potato peel showed the TFC of (0.7 mg/g). When extracted with 80% aqueous methanol as a solvent. (Mohamad and Fatma, 2010) The results were in agreement with who studied the process for extraction of antioxidants from peel, but differed slightly this may be due to the experimental and environmental conditions. The concentration of phenolics and flavonoids in the extracts, expressed as mg of GAE/g sample was dependent on the solvent and method used in the extraction. The amount of phenolic compound in the ethanolic extract was highest and total phenolics concentration in the two solvents were in the order: Ethanol > 80% aqueous methanol. Peels contain many phenolics compounds, some are in free form and some are in bound form. The major phenolics acid in the peel extract were identified as chlorogenic acid (CGA), gallic acid (GAC), protocatechuic acid (PCA), and caffeic acid (CFA).

Total antioxidant activity

Free radicals involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases. DPPH is considered to be a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is initiated by lipid autoxidation. Antioxidants react with DPPH, reducing the number of DPPH• free radicals to the number of their available hydroxyl groups. Therefore the absorption at 515 nm is proportional to the amount of residual. The extracts that showed relatively high antioxidant activity (those with methanol and ethanol), as strong as that of BHA and BHT but weaker than that of TBHQ, contained the highest amount of total phenolic compounds.

The results of the DPPH free radical-scavenging assay suggest. That components within the extracts are capable of scavenging free radicals via electron-or hydrogen-donating mechanisms and thus should be able to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices, e.g. biological membranes. This further shows the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage. DPPH. It is visually noticeable as a discolouration from purple to yellow. The scavenging activity of extracts against DPPH was concentration-dependent. Significant ($P < 0.05$) differences between extracts were observed, but the results clearly indicate that all extracts exhibited antioxidant activity. The extracts that showed relatively high antioxidant

CONCLUSION

Antioxidant activity of different peels have health promoting and disease-preventing effects. Consequently, consumption of eggplant, beetroot and potato which contains polyphenols, from eggplant, beetroot and potato may have a potential therapeutic use. This study confirms that the phenols and flavonoids are present in various parts of vegetables used, particularly in skin, suggesting the use of the entire vegetable as food. Extraction may be an attractive alternative to conventional method to improve the amount of polyphenols and the antioxidant activity extracted, in particular for nutraceutical application. Extraction of skin with ethanol was particularly effective as compared to 80% aqueous methanol.

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