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Determination of total phenolic content in different vegetables

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Abstract
The word polyphenol is that chemical substance having more than one phenol group. Polyphenols represent a variety of compounds of plant origin. Polyphenols have potential use in the human body. They act as antioxidants preventing oxidation of other molecules by formation of free radicals in the body. Most of the polyphenols have properties of reducing agents. They protect cell constituents against oxidative damage. Therefore polyphenols reduce risk of various degenerative diseases related to oxidative stress. In addition to antioxidant properties, they also have antimicrobial as well as anti-inflammatory properties. They enhance body immunity. Polyphenol intake or consumption of polyphenol-rich foods has been studied in several epidemiological studies. Many studies on the antioxidant properties of vegetables suggested that vegetables are excellent dietary sources of natural antioxidants. Polyphenols are natural antioxidants present in all foods. The study was conducted to determine total phenolic content in various vegetables. Total phenolic content estimated in vegetables were compared. Total phenols are estimated by Folin Denis reagents that produce blue color with polyphenols. Tannic acid was used as standard and results were expressed as tannic acid equivalent (Singh and Jambunathan, 1981). Result: Total polyphenol contents in present study showed variation within vegetables estimated. Mean polyphenol content in vegetables was found to be highest 5.3g/100g in amla, followed by mint 1.1g/100g, 844 mg/100g in coriander green and 586 mg/100g in lady finger. Amla was characterized as the highest polyphenol content (538 mg/100g) on a fresh weight basis, whereas the lowest mean polyphenol content (17.50mg/100g) was found in cucumber. Conclusion: fruits and vegetables must be included in our daily diet for good health.

Keywords: Polyphenols, antioxidants, vegetables, total polyphenol content, ageing, folin denis reagent, spectrophotometer, optical density

Introduction
Free radicals or reactive oxygen species (ROS) are formed in the body as a result of oxidation. Oxidation by products of normal metabolism causes damage to the body resulting in degenerative diseases. Antioxidant defense mechanism diminishes action of free radicals. Sources of natural antioxidants are mainly plant phenolic compounds.

The term polyphenols means with chemical compounds that have more than one phenolic group. They are commonly known phytochemicals, found abundantly in plant foods. Polyphenols have antioxidant properties. They prevent oxidation in cells by the formation of free radicals. Hence protect cells from aging. In addition to this they have antimicrobial, anti-carcinogenic and anti-inflammatory properties. Keeping in view the beneficial health effects of polyphenols, several researches have been conducted in recent years all over the world.

These compounds can be classified into various groups depending on their chemical structure. Polyphenols are mainly grouped into phenolic acids, flavonoids, stilbenes and lignans. Phenolic acids can be divided into two subclasses: derivatives of benzoic acids and derivatives of cinnamic acids. They are known as hydroxybenzoic acids and hydroxycinnamic acids. The hydroxybenzoic acid (protocatechuic acid and gallic acid) content of edible plants is very low, except certain red fruits, black radishes and onions (Shahidi and Naczk, 1995) [15]. Hydroxybenzoic acids are found only in a few edible parts of plant foods. So, they have not been extensively studied. Hydroxycinnamic acids (coumaric, caffeic acid, ferulic acid) are more common than hydroxybenzoic acids. They are found in processed foods i.e. frozen,

sterilized or fermented foods.

Flavonoids are subdivided into flavonols, flavones, flavanones, isoflavones, flavanols, proanthocyanidins and anthocyanins. Flavonols are generally present in low concentration i.e. about 15-30 mg/Kg on fresh weight. Richest sources of flavonols are onions, chocolates, leeks, broccoli and blueberries. So these compounds accumulate in outer or aerial parts of fruits and vegetables. Flavones are present in lesser amounts than flavonols in fruits and vegetables. Cereals such as millets and wheat also have flavones in them. Flavonones are mainly found in tomatoes and mint. Citrus fruits have higher concentration of flavonoids. Isoflavones are flavonoids having similarities to estrogens, but they are not steroids. They have pseudo-hormonal properties. So, they are called phytoestrogens. Rich sources of isoflavones are leguminous plants such as soya. Flavanols are found in the form of catechins and proanthocyanidins. Major sources of catechins are green tea, red wine and chocolates. Anthocyanins are pigments that impart pink, red, blue or purple color to certain fruits and vegetables. Colored or colorless properties of plant foods depend on the content of anthocyanins present in them. These compounds are abundantly present in fruits, red wine, certain varieties of cereals and some leafy as well as root vegetables (cabbage, beans, onions, radishes). The richest source of lignans in foods is linseed. Cereals, grains, fruits and vegetables are fair sources of lignans. Stilbenes are rarely found in the human diet. One of them: Resveratrol has anti-carcinogenic properties, but it is present in smaller amounts in diet.

Good sources of polyphenols in the human diet are fruits, vegetables and beverages (Cieslik *et al.* 2006; Kaur and Kapoor, 2001) [2, 7]. Composition of polyphenols in plant products depends on their varieties and edible parts. Some other factors i.e. harvesting, environmental factors, processing and storage also affect polyphenol content in plant foods. Environmental factors are mostly responsible for the content of polyphenols in foods. These factors include soil type, sun exposure, rainfall, greenhouse, fruit yield per tree. Sunlight has a major effect on most flavonoids, the ripening period affects concentration and proportion of various polyphenols. Phenolic content in plant foods decreases as the fruit ripens, while anthocyanins concentration increases after ripening. It has been noticed that polyphenol content in vegetables produced by organic or sustainable agriculture is found to be higher than conventionally grown ones. Storage also affects polyphenol content. This is due to oxidation reactions in them, leading to change in color and organoleptic features. Quantity of polyphenol content in cereals is lowered up to 70% after 6 months of storage while cold storage does not affect their content in fruits and vegetables. Peeling of fruits and vegetables eliminates a significant portion of polyphenols, as they are generally present in higher concentration in outer layers than inner layers.

Polyphenols have potential use in the human body. They act as antioxidants preventing oxidation of other molecules by formation of free radicals in the body. Most of the polyphenols have properties of reducing agents. They protect cell constituents against oxidative damage. Therefore polyphenols reduce risk of various degenerative diseases related to oxidative stress. In addition to antioxidant properties, they also have antimicrobial as well as anti-inflammatory properties. They enhance body immunity. Polyphenol intake or consumption of polyphenol-rich foods has been studied in several epidemiological studies.

It has been found that moderate consumption of tea and wine lowers risk of myocardial infarction in both cases control and cohort studies (Peters *et al.* 2001) [11]. Higher intake of polyphenols is likely to have beneficial effects on health, preventing the body against cardiovascular disease, diabetes, neurodegenerative diseases and cancer (Scalbert *et al.* 2005). Polyphenols also inhibit platelet aggregation *in vitro* (Russo *et al.* 2001) [12]. Most polyphenols help against prevention of cancer. They cause cancer cells to die. These compounds repair D.N.A. and damage caused by smoking and other toxic exposures reduce the number of tumors or their growth (Yang *et al.* 2001) [17].

Methodology

Estimation of polyphenols in foods

Principle: Folin Denis reagent produces blue color with polyphenols. Tannic acid was used as standard and results were expressed as tannic acid equivalent (Singh and Jambunathan, 1981) [16].

Reagents

1. **Folin Denis reagent**
   100 g sodium tungstate, 20 g phosphomolybdic acid, 50ml phosphoric acid were added to 750 ml distilled water and refluxed for 2 h, cooled and diluted to 1 liter.
2. **Saturated sodium carbonate**
   45 g anhydrous sodium carbonate was dissolved in 100 ml distilled water at 70º-80º C and cooled. Supersaturated solution with sodium carbonate crystals was filtered through glass wool.
3. **Tannic acid**
   100 mg tannic acid was dissolved in distilled water and volume was made to one liter with water. Solution was prepared fresh for each determination.
4. **Methanol-HCl**
   10 ml conc. HCl was mixed in 100 ml methanol and volume was made to one liter with methanol.

Sample selection and preparation

Sample of each food was purchased from the local market of Rohtak city of Haryana**.** All the vegetables were bought fresh and no canned, frozen and tinned foods were used for estimation. Samples were cleaned; edible parts were removed in vegetables analyzed immediately. Each sample was taken in triplicate and mean was reported. Polyphenolic compounds were extracted from vegetables on a dry/fresh weight basis using Folin Denis reagent.

Extraction

Different samples were weighed and then homogenized. 200mg defatted homogenized sample was taken in 250 ml round bottom flask and refluxed with 100 ml methanol-HCl for 2 hours, was allowed to cool. Extract was filtered through whatman no. 40 filter paper in 100 ml volumetric flask. Volume was made with methanol-HCl after a few washing. 0.2 ml extract was taken for estimation of polyphenols. The amount of polyphenolic compounds were estimated as tannic acid equivalent according to the Folin- Denis procedure (Swain and Hills, 1959).

Estimation

0.2 ml of the extract was diluted with 7.5 ml water in 10 ml volumetric flask. Standard tannic acid of different concentrations ranging between 0.2 to 1 ml was taken. 0.5 ml Folin Denis reagent was added to blank, standard and extract and shacked. One ml of saturated sodium carbonate solution

was added to all (blank, standards and sample). Volume was made to 10 ml with distilled water. Vortexed thoroughly and allowed to stand for 30 minutes. Color was stable for up to 40 minutes. Absorbance was read in the spectrophotometer at 760 nm using a suitable blank. A standard curve was plotted by taking different concentrations of tannic acid, 0.300 O.D. corresponds to 60µg tannic acid. Suitable volumes of sample were taken to fit into standard concentrations in the standard curve.

Result and Discussion

The present study was carried out to determine total phenolic content in various vegetables using a folin-denis reagent and optical density of the sample was measured by drawing a standard curve.

Polyphenol content in vegetables

The mean polyphenol content in vegetables tested in present study ranged between - 17.50 to 5380.00 mg/100g on a fresh weight basis.

Table 1: Polyphenol content in Vegetables

Food group	Name of food stuff	Total polyphenol content(mg/100g)
Vegetables FW	Potato	24.14 ± 11.38
	Tomato	52.60 ± 12.89
	Onion	58.30 ± 8.30
	Fenugreek leaves	323.70 ± 12.75
	Capsicum	70.93± 8.77
	Carrot	62.30 ± 5.87
	Cauliflower	77.22 ± 9.55
	Cabbage	107.10 ± 0.10
	Cucumber	17.50±3.20
	Brinjal	343.72 ± 9.13
	Spinach leaves	244.55 ± 19.29
	Lady finger	586.80 ± 19.91
	Bottle gourd	143.83 ± 17.35
	Coriander green	844.81 ± 38.40
	Beet root	341.40 ± 27.57
	Mint	1110.00 ± 45.80
	Pumpkin	18.63 ± 8.78
	Amla	5380.00 ± 26.70

Values are mean ± SD
FW fresh weight basis

Total polyphenol contents in present study showed variation within vegetables tested (Table 1). Mean polyphenol content in vegetables tested was found to be as 5380.00±26.70mg/100g in amla, 1110.00±45.80mg/100g in mint, 844.81± 38.40mg/100g in coriander green and 586.80± 19.91mg/100g in lady finger. Brinjal had polyphenol content 343.72 ± 9.13mg/100g, beet root had 341.40± 27.57mg/100g, fenugreek leaves had 323.70±12.75mg/100g and spinach leaves had 244.55± 19.29mg/100g. The mean polyphenol content in bottle gourd was 143.83±17.35mg/100g, in cabbage 107.10±0.10mg/100g, in cauliflower 77.22± 9.55mg/100g, in capsicum 70.93±8.77mg/100g, in carrot 62.30±5.87mg/100g, in onion 58.30±8.30mg/100g, in tomato 52.60±12.89mg/100g, in potato 24.14± 11.38mg/100g and in pumpkin was 18.63 ±8.78mg/100g on fresh weight, results are arranged in ascending order. Amla was characterized as the highest polyphenol content (5380.00 ± 26.70mg/100g) on a fresh weight basis, whereas the lowest mean polyphenol content (17.50±3.20mg/100g) was found in cucumber.

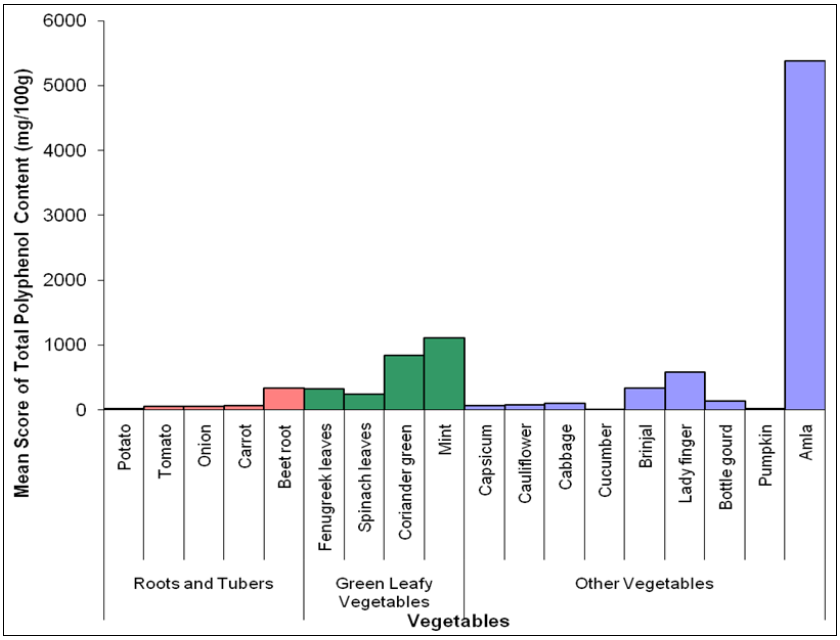

Fig 1: Polyphenol content in vegetables

Similar results have been reported by Zujko and Witkowska (2011) [18]. Polyphenol content in fruits and vegetables was assessed by Zujko and Witkowska (2011) [18]. The mean polyphenol content among fruits and vegetables ranged from 72±4 to 239 ± 89 mg/100g and 17±3 mg/100g to 283±69 mg/100g, respectively. Berries and grapes were found to be the richest sources of polyphenols. Fennel and red cabbage had the highest content of polyphenols, while cucumber had the lowest content of polyphenols.

Conclusion

It can be concluded from a present study that vegetables have high polyphenolic content. On the Basis of the beneficial effects on health, it can be suggested that vegetables having high polyphenol content in their recipes can be developed. Indian council of medical research expert report recommended intake of 400 g of fruits and vegetables in the Indian diet per person per day to meet the requirement of antioxidants (ICMR, 2010). Intake of vegetables in Indian diets must be increased.

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