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Estimation Of Bioactive Components In Indian Cereals And Pulses

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ABSTRACT

Background: Cereals and pulses are primary staple foods and often contribute a major portion of daily human energy and protein intake all over the world. Consuming whole grain cereals and pulses showed protective effects against various chronic diseases as documented in various research articles. However, potential benefits of combined intake of whole cereals and pulses about amino acid nutrition are rarely contributed in literature. There is significant evidence which proves that key bioactive components of whole grain cereals and pulses are structurally different and hence may be optimized to provide complementary health benefits. Among the most common whole grain bioactive components are polyphenols, vitamins, minerals and dietary fiber. Polyphenols are the most common natural antioxidants in the human diet. They are the phenolic compounds that are widely spread in all plant foods. The polyphenols are divided into three major classes mainly phenolic acids, flavonoids and tannins. These compounds have beneficial health effects related to their antioxidant (Vinson *et al.* 2005), anti-inflammatory properties, cardio-protective, chemo-preventive and neuro-protective properties. Due to their health promoting and disease-preventive properties, polyphenols are also called functional foods. **Objective:** The study was conducted to determine total phenolic content in various cereals and pulses. Total phenolic content estimated in cereals and pulses were compared. **Materials and Methods**: Total phenols are estimated by Folin Denis reagent that produces blue color with polyphenols. Tannic acid was used as standard and results were expressed as tannic acid equivalent (Singh and Jambunathan, 1981). **Result and Discussion:** Polyphenol content in cereals and pulses has been determined. Bajra flour contains the highest content of polyphenols 640.66±121.50 mg/100g tannic acid equivalent on a dry weight basis as compared to rice and wheat flour. The lowest polyphenol content was found in rice. Mean polyphenol content in pulses ranged between 73.66-1303mg/100g. Green gram whole had the highest (1303 mg/100g) polyphenol content, whereas green peas had the lowest (73.66mg/100g). Total polyphenol content in the present study showed variation within cereals and pulses estimated. **Conclusion:** Keeping in view health benefits of polyphenols as natural antioxidants, antioxidants rich diet must be consumed widely in daily.

Keywords: Polyphenols, Antioxidants , Cereals, Pulses, Total polyphenol content, Ageing, Folin Denis reagent, Spectrophotometer, Optical Density, Polyphenols

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1. INTRODUCTION

Cereals and pulses are primary staple foods and often contribute a major portion of daily human calorie and protein intake all over the world. Consuming whole grain cereals and pulses showed protective effects against various chronic diseases as documented in various research articles. However, potential benefits of combined intake of whole cereals and pulses about amino acid nutrition are rarely contributed in literature. There is significant evidence which proves that key bioactive components of whole grain cereals and pulses are structurally different and hence may be optimized to provide health benefits.

Cereal and pulses are the major sources of calories and protein in the human diet. In addition to energy and protein, They are also good sources of different bioactive compounds, vitamins (B1, B2, Pantothenic acid, Niacin) , dietary fiber (β-glucan) and minerals. Most of the existing studies have focused on the bioactive compounds in fruits, vegetables and beverages. Some of the bioactive compounds are unique in nature and only present in cereals but cannot be obtained from fruits and vegetables (Jones et. al., 2004).Cereals and pulses generate valuable byproducts during their processing treatments i.e. dehulling, milling which are a good source of potentially bioactive compounds. These byproducts are intensely utilized in extraction of the bioactive compounds such as polyphenols, vitamins, flavonoids, dietary fibers etc. (Carciochi et. al., 2017).

Bioactive components in Cereals and Pulses

Bioactive compounds are mainly present in small quantities whereas their chemical structures, properties and functions variable.These compounds are synthesized after primary metabolism (e.g.amino acids) or by their intermediates obtained during primary metabolism in specialized cells depending on types and only during stage of growth,or under specific conditions i.e. their extraction and purification. The commercial useful bioactive compounds are generally secondary metabolites i.e. are terpenoids, polyphenols, vitamins, alkaloids, which are utilized in the development of functional foods and pharmaceuticals.

- **Carotenoids**
- **Phytic acid and Oxalic acid**
- **Saponins**
- **Phytosterols**
- **Polyphenols/Phenolic acids**

Carotenoids

Carotenoids are a class of more than 600 naturally occurring organic pigments synthesized by plants. These compounds provide a source of color like yellow, orange, and red (Britton et. al, 2004) and are important because of their role in synthesis of pro-vitamins and antioxidants. These compounds are classified into two classes, carotene (β-carotene, γ-carotene, α-carotene and β-cryptoxanthin) and xanthophylls. Carotenoids are present in whole-grain cereals (Adom et. al, 2005) whereas fruits and vegetables are the principal sources of carotenoids and its deficiency may cause different diseases related to malfunctioning in eyes i.e. night blindness, keratomalacia, xerophthalmia, corneal ulceration, scarring and at last blindness (Vucenik and Shamsuddin,2006).

Phytic and oxalic acid

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Phytic acid is the major source of phosphorus and its salts known as the phytates which can result in various health beneficial effects having anticarcinogenic properties and decreases the risk of heart diseases or diabetes (Minihane M and Rimbach, 2002). Phytic acid is an important seed constituent, which is present in all nuts, aleurone layer of cereals, legumes and oilseeds(Sandberg, 2002) . Oxalic acid is present in the cell sap of the plants in the form of Potassium or Calcium salts. Legumes are a good source of minerals but their bioavailability was observed lower than other food due to the phytates and oxalates (Normen, et. al, 2001).

Phytosterols

Phytosterols are the combination of plant sterols and stanols that are present in smaller quantities. Their structures are similar to cholesterol, differ only in the side chain group and/or presence or absence of the double bond. The most common phytosterols are β-sitosterol, stigmasterol and campesterol. These compounds are present in different legumes i.e. kidney bean, pea, chickpea. Cereal products contain a significant amount of plant sterols than fruits and vegetables. These compounds vary in different cereals and in various parts of the kernel (Liu, 2007). Various studies have shown that sterols and stanols are beneficial in preventing colon cancer and decreasing the cholesterol level (Oakenfull, 1981).

Saponins

The 'saponin' word is derived from the Latin word sapo that means 'soap', because saponin produces soap-like foams when shaken with water. These molecules are known as non-volatile, Saponins have been determined in lupin, lentil, pea, black gram and beans, whereas major sources of saponins are soybean and chickpea in the human diet (El-Adawy,2002). Various studies suggest that saponins possess anti-carcinogenic and hypocholesterolemic properties.

Polyphenols/Phenolic acids

Phenolic compounds are commonly known for their antioxidant properties and protection from degenerative diseases i.e. cancer and heart diseases. Phenolic acids are a group of the derivatives of benzoic and cinnamic acid. Flavonoids are the compounds that consist of two aromatic rings joined by a three-carbon link. Flavonoids are categorized into different sub-classes such as anthocyanins, flavonols, flavanones, flavones and flavonols. Moreover, lignans are a group of the dietary phytoestrogens that are present in cereals like wheat, oat, and corn.

Classification of polyphenols

The word polyphenol means chemical substance having more than one phenol unit. Polyphenols represent a wide variety of compounds of plant origin. Several thousand compounds of polyphenol structure have been identified in plants till date. These compounds can be classified into various groups depending on their chemical structure. Polyphenols are mainly grouped into phenolic acids, flavonoids, stilbenes and lignans.

Fig. 1 Classification of Polyphenols

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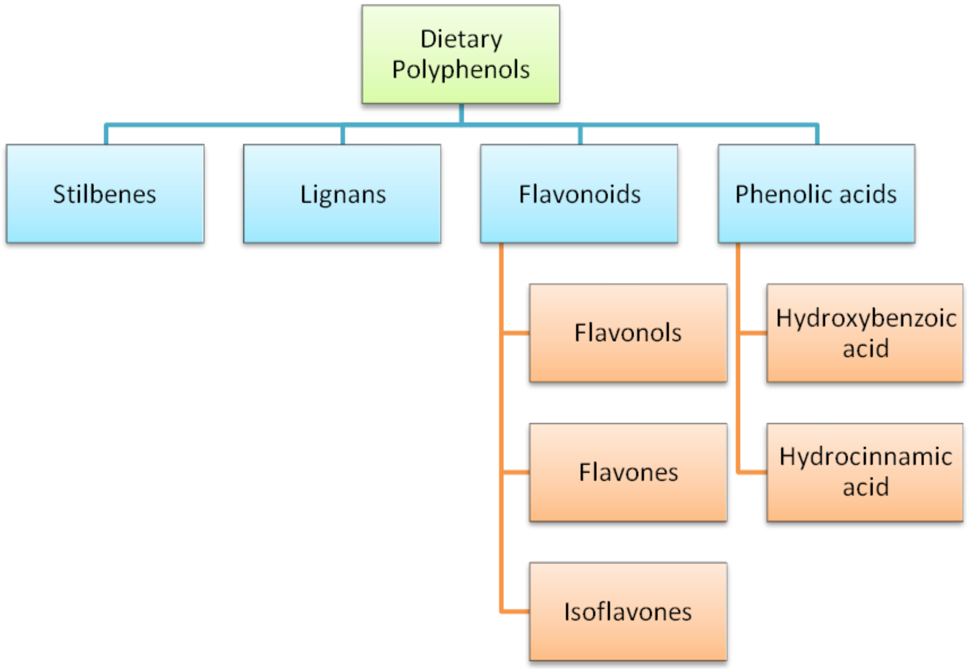

Phenolic acids can be divided into two subclasses: derivatives of benzoic acids and derivatives of cinnamic acids. They are known as hydroxybenzoic acids and hydroxycinnamic acids. The hydroxybenzoic acids (protocatechuic acid and gallic acid) content of edible plants is very low, except certain red fruits, black radishes and onions (Shahidi and Naczk, 1995). Tea is an important source of hydroxybenzoic acids (Gallic acid) containing up to 4.5g/Kg fresh weight (TomasBarberan and Clifford, 2000). 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. Blueberries, kiwis, plums, cherries and apples contain the highest amount of hydroxycinnamic acid ranging from 0.5-2g/Kg on fresh weight. Caffeic acid commonly known as phenolic acid is present in most of the fruits. These compounds are generally present in the outer layer of ripened fruits. Concentration of hydroxycinnamic acids decreases as fruit undergoes ripening and quantity increases as the size of fruit increases (Manach *et al.* 2004). Cereals contain hydroxycinnamic acids in the form of ferulic acid which is about 0.8 -2g/Kg on dry weight and represents 90% of total polyphenols.
Ferulic acid is present in outer layers of grains. Thus its content is directly related to the extent of sieving. Bran accounts for most of the polyphenols in cereals.

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. Red wine and tea contain up to 45 mg flavonols/L and their synthesis is stimulated by light. 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

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mint. Citrus fruits have higher concentration of flavonones. Isoflavones are flavonoids having similarities to estrogens, but they are not steroids. They have pseudo-hormonal properties. So, they are called phytoestrogen.

Health Benefits of Polyphenols

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.

Fig. 2 Health Benefits of Polyphenols

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). 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). 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). Neurodegenerative diseases as Alzheimer's and Parkinson's disease are found to be dependent on oxidative stress which are associated to brain tissues

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(Halliwell, 2001) and antioxidants may contribute to prevention of these diseases (Canturi-Castelvetri *et al.* 2000). They also help in prevention of diabetes (Zunino *et. al.* 2007).

Some studies have shown adverse effects of polyphenols on health due to their anti-nutritional properties. Most of the dietary polyphenols have catechol group in their structures and thus form very stable chelates with ferric ions. They are only observed when a polyphenol rich source is ingested with a source of iron. Polyphenol containing beverages such as tea and coffee inhibit non-heme iron absorption, as shown in clinical trials (Hurrell *et al.* 1999). They interfere with thyroid hormone biosynthesis.

2. Review of Literature

Sreeramulu *et al.* (2009) determined antioxidant activity (AOA) and phenolic content (PC) in commonly consumed cereals, millets, pulses and legumes in India. AOA was estimated by 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and reducing power. DPPH activity ranged from 0.24 and 1.73mg/g, whereas FRAPS ranged from 16.21 to 471.71micromoles/g. Finger millet and rajma had the highest FRAP 471.71, 372.76 and DPPH activity 1.73, 1.07. Similar results were found in reducing power. Finger millet and black gram dhal had the highest phenolic content 373 and 418mg/100g, respectively, whereas rice and green gram dhal had the lowest phenolic content 47.6 and 62.4mg/100g, respectively.

Zujku and Witkowska (2011) determined polyphenol content in selected foods. Total antioxidant potential was estimated with Ferric Reducing Antioxidant power method. Total polyphenol content of 44 commonly consumed foods was determined using the folin-ciocalteu method. Absorbance was read after 30 minutes at 765 nm on spelcol 10 spectrophotometer. Antioxidant potential of foods tested was related to total polyphenol content. Total polyphenol content and antioxidant potential varied for vegetables: 17-283 mg/100g and 0.033-3.209mmol/100g fresh mass, for pulses: 142-191 mg/100g and 0.342-0.387mmol/100g fresh mass, for fruits: 72-239mg/100g and 0.312-2.833mmol/100g, for cereals: 42-327 mg/100g and 0.062-1.709mmol/100g fresh mass, respectively. Food products such as funnel, red cabbage, strawberries and buckwheat groats were reported to be good sources of polyphenols, should be recommended for consumption.

Ryan *et al.* (2011) analyzed and compared polyphenol content and total antioxidant activity of 30 commercially grown oat based breakfast cereals. All breakfast cereals analyzed were a significant source of polyphenols (1506-1853µg gallic acid equivalent (GAE/g).The polyphenol levels in an average serving of 40g oat based breakfast was found to be comparable to those found in fruits and vegetables.

Sreeramulu *et al.* (2013) determined antioxidant activity and phenolic content in commonly consumed plant foods in India. Highest phenolic content was observed in areca nut 1084 mg Gallic Acid Equivalent /100g, whereas lowest was reported in coconut water 10 mg/100g. Values of phenolic content in cereals/millets ranged from 47 to 373 mg/100g. Finger millet had the highest phenolic content 373 mg/100g and the lowest phenolic content was reported in milled rice 47mg/100g. Phenolic content in the fresh fruits ranged from 26 to 374 mg/100g being highest in guava and lowest in watermelon. Curry leaves had the highest (1077 mg/100g) phenolic content and the least was found in spinach (77 mg/100g). Roots and tubers showed a wide range of phenolic content 22 to 339 mg/100g, which was highest in beetroot and lowest in

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carrot. Among sugars and oils jaggery had the highest phenolic content 336 mg/100g and least (0.72mg/100g) was found in vanaspati.

Fratianni *et al.* (2014) estimated phenolic contents and antioxidant activity of green pea, lentil and chickpea from southern Italy. Lentil had the highest antioxidant activity (EC_{50} not higher than 2.3mg/g). Green peas tested exhibited lower content of total polyphenols and tannins as compared to other grass peas. Chickpeas (cv Castelcivita and Sassano) exhibited the lowest content of total polyphenols (147.13 and 183.78 µg/g of raw product, respectively). These legumes can be considered as therapeutic functional food due to their significant content of functional protein, carbohydrates and bioactive constituents. They are beneficial for managing and preventing severe chronic illness in humans.

3. Methodology:

Estimation of polyphenols in Cereals and Pulses

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).

Reagents:

(i) 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.

(ii) 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.

(iii) 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.

(iv) 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 item was purchased from the local market of Rohtak city of Haryana**.** All the cereals and pulses bought fresh were used for estimation. Samples were cleaned; edible parts of cereals and pulses analyzed immediately. Each sample was taken in triplicate and mean was reported. Polyphenolic compounds were extracted from cereals and pulses on a dry/ weight basis using Folin Denis reagent.

Extraction

Different samples were weighed and then homogenized. A 200 mg defatted homogenized sample was taken in a 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

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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.

4. Result and Discussion

Polyphenol content in cereals and pulses

Polyphenol content in cereals and pulses has been projected in Table 1. Bajra flour contains the highest content of polyphenols 640.66±121.50 mg/100g tannic acid equivalent on a dry weight basis as compared to rice and wheat flour. Mean polyphenol content in rice and wheat flour were 75.00±20.50mg/100g and 207.66 ± 6.80mg/100g on a dry weight basis. The lowest polyphenol content was found in rice.

Zujko and Witkowska (2011) reported total polyphenol content in cereal products, which was found the highest (327 ± 41 mg/100g in fresh mass) in buckwheat groats and the lowest in rice (42±10 mg/100g). Mean total polyphenol content in wheat flour was found to be 110±20 mg/100g in dry mass. The results were expressed in terms of Gallic Acid Equivalents (GAE). The polyphenol content in oat based cereals ranged from 1506 to1853µg gallic acid equivalent/g (Ryan *et al.* 2011). Polyphenol content in cereals/millets ranged 47-373 mg/100g GAE (Sreeramulu *et al.* 2013). Finger millet had the highest phenolic content 373 mg/100g, whereas it was found to have the lowest 47 mg/100g GAE in milled rice.

Findings of present study are different from others; this variation could be due to the fact that all the researchers used gallic acid as standard whereas in the present study tannic acid was used as standard. To compare our findings on natural polyphenol contents on cereals, there is very little published data available in India. Another reason for the difference in findings might be due to the fact that polyphenol content in foods depends on soil composition, crop variety, plant genetics, storage, harvest conditions, post harvest conditions and climatic conditions.

Table 1 Polyphenol content in Cereals and Pulses

S.No.	Food group	Name of food stuff	Total Polyphenol content in dry weight (mg/100g)
1	Cereals	Wheat flour whole	207.66 ± 6.80
		Rice	75.00 ± 20.50
		Bajra flour	640.66 ± 121.50
2	Pulses	Bengal gram whole	137.70 ± 10.32
		Bengal gram dhal	250.00 ± 50.00
		Black gram whole	1283.00 ± 66.50
		Black gram washed	225.00 ± 35.35
		Cow pea	136.33 ± 45.50
		Green gram whole	1303.00 ± 90.10
		Green gram washed dhal	75.00 ± 21.30
		Lentil	217.60 ± 23.50
		Red gram dhal	309.27 ± 145.79

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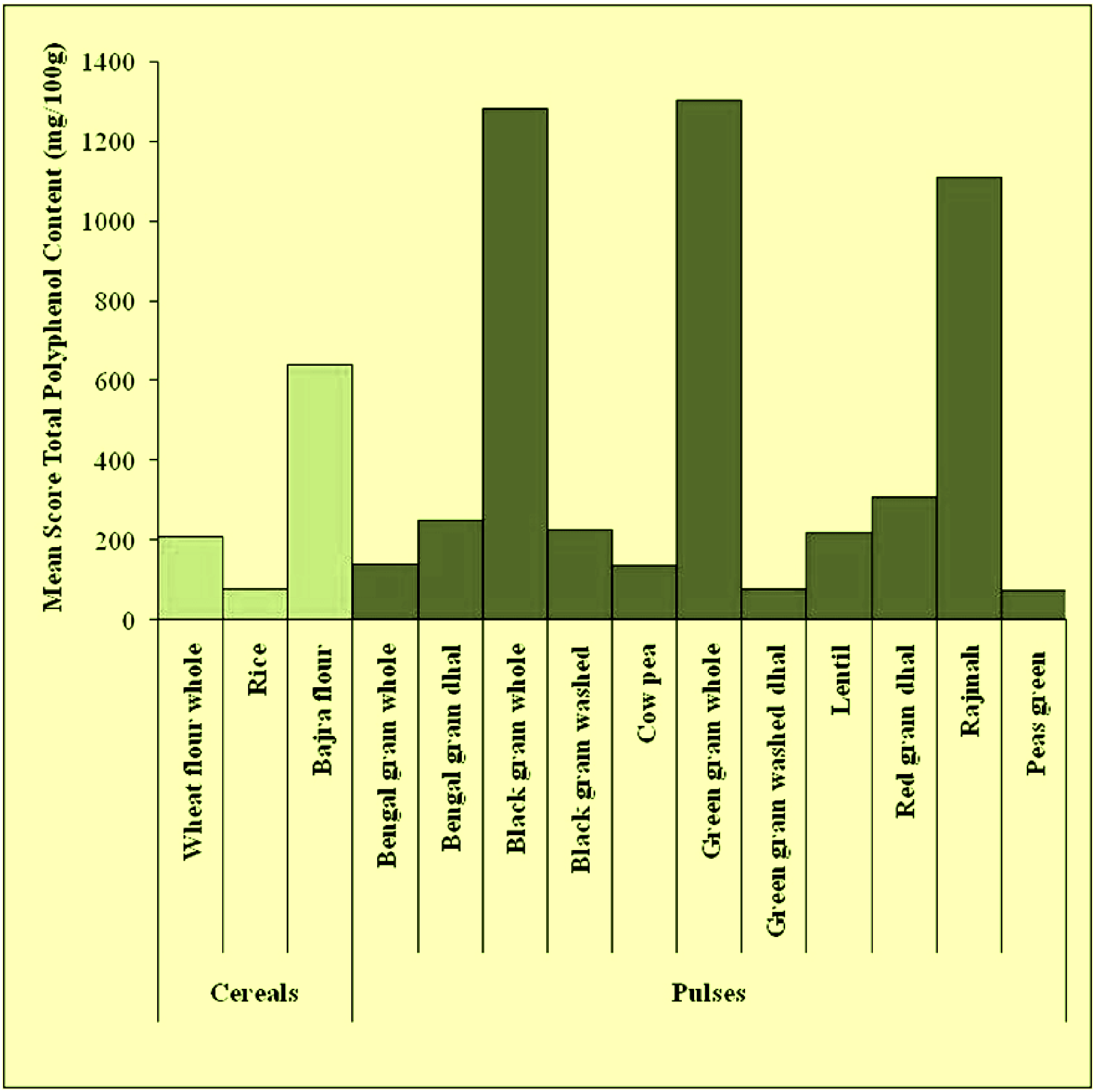
		Rajmah	1110.00 ± 98.48
		Peas green	73.66 ± 11.57*

Values are Mean ± SD
*polyphenol content in fresh weight

Fig. 3: Polyphenol content in Cereals and Pulses



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Mean polyphenol content in pulses ranged between 73.66-1303mg/100g. Green gram whole had the highest (1303 mg/100g) polyphenol content, whereas green peas had the lowest (73.66mg/100g). Mean polyphenol content was 1283.00±66.50mg/100g in black gram whole, 1110.0±98.48 mg/100g in rajma, 309.27 ± 145.79mg/100g in red gram dhal, 250.00 ± 50.00 mg/100g in bengal gram dhal, 225.00 ± 35.35mg/100g in black gram washed and was 217.60±23.50 mg/100g in lentil. Bengal gram whole contained polyphenol content 137.70±10.32 mg/100g, whereas cow pea and green gram washed contained 136.33± 45.50mg and 75.00 ± 21.30mg/100g on dry weight basis, respectively. Mean polyphenol content in green peas was 73.66±11.57mg/100g on a fresh weight basis. Among pulses, green gram whole was found to be the richest polyphenol content followed by black gram and rajma. It can be concluded that whole pulses contain more polyphenols than dehusked, as seed coats contain more polyphenols.

The mean polyphenol content in peas and beans was 191±26 mg/100g and 142 ± 20 mg/100g in fresh mass, respectively (Zujko and Witkowska, 2011). Among the pulses and legumes, values of polyphenol content ranged from 62-418 mg/100g, black gram dhal had the highest (418 mg/100g), while green gram dhal had the least (62 mg/100g) as reported by Sreeramulu *et al.* (2013). Chickpeas varieties contained total polyphenol content as 147.13 and 183.78µg/g of raw product (Fratianni *et al.* 2014).

Polyphenol content in green gram dhal was comparable with present findings as reported by Sreeramulu *et al.* 2013. Variation in results could be due to the fact that all the researchers used gallic acid as standard whereas in the present study tannic acid was used as standard. Pulses had a wide range of polyphenol compounds located in their seed coat and also contributed to antioxidant capacity (Duenas *et al.* 2003).

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