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BIOCHEMICAL ANALYSIS OF WHEAT, BARLEY, FOR HEALTH COMPONENTS IN GRAIN

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ABSTRACT:

The presents Studies, Some of the latest biochemical research endeavors that aim to improve our understanding of how the various grain components can be manipulated to improve contributions of cereals to human health. The health benefits of wholegrain cereal products are now widely recognized and considered due to the presence of a wide range of bio-active components. Cereals in their natural form (as whole grain) are rich source of vitamins, minerals, carbohydrates, fats, oils, and protein. Also, such cereals are chosen for consumption and they have the higher fiber which is an important nutrient that helps to prevent weight gain and heart disease. For best results, fruits, vegetables and whole grains may be eaten every day to provide extra nutrition and fiber. The meal can be made healthier by eating high-fiber whole-grain cereals that has low sugar. However, eating a variety of cereals rather than just feeding on one item is more helpful to health.

Key words: *Cereal, grain, human, health, diet,*

INTRODUCTION

The term cereal is a derivative from Latin word 'cerealis' meaning ‘grain’ which is botanically, a type of fruit called a caryopsis, composed of the endosperm, germ, and bran. The cereals are annual common grass members of the grass family (a monocot family Poaceae, also known as Gramineae), which usually have long, thin stalks, such as wheat, rice, maize, sorghum, millet, barley and rye, whose starchy grains are used as food. The term cereal is not limited to these grains, but, also refers to foodstuff prepared from the starchy grains of cereal like flours, breads and pasta. Cereal science is a study concerned with all technical aspects of cereal. It is to study the nature of the cereals and the changes that occur naturally, and as a result of handling and processing.

Cereals constitute the major source of calories for humans as rice in India and the Far East, maize in Mexico and wheat and barley in Europe and North America. Staple food grains are often called corn.

Materials AND Methods

Present investigation was undertake to analyse some biochemicals in five different selected cereals seedlings of the family Poaceae under different ages. Their seeds were obtained from local supplier - National Seed Corporation (NSC).

Selection of plants

Considering the literature, five members of family Poaceae were selected for the present study.

1. Wheat – *Triticum aestivum* L.
2. Corn – *Zea mays* L.
3. Barley – *Hordeum vulgare* L.
4. Oat – *Avena sativa* L.
5. Rice – *Oryza sativa* L

Biochemical Parameters

1. Proteins
2. Carbohydrates
3. Nutrients

(I) Nitrogen

(II) Phosphorus

(III) Calcium

(IV) Iron

(V) Magnesium

(VI) Potassium

(VII) Sodium

(VIII) Zinc

Experimental Studies

Protein

Protein estimation in different plant organs leaves/root/seeds was carried out according to method

developed by Lowry (1951). Fresh tissue (50 mg) was homogenised in 5 ml chilled tris maleate buffer and centrifuged at 2000 rpm for ten minutes. To 1 ml of supernatant 1 ml of TCA (20%) was added and kept in refrigerator for overnight. Next day, reaction mixture was again centrifuged and pellet was dissolved in 0.1N NaOH (3 ml). After 2 h. 1 ml of above solution was mixed with 5 ml alkaline copper tartarate solution. Ten minutes later, 1 ml of folin ciocalteau reagent (double diluted) was added and solution was kept in dark for 30 min. Then optical density (OD) of sample was recorded at 660 nm and amount of protein (mg g^{-1} fresh weight) was determined with the help of standard curve of bovine serum albumin (Fig. no - 1).

Carbohydrate

Total carbohydrate amount was estimated by anthrone colorimetric method (Yemm and Willis, 1954). In this method plant leaves were placed in an oven for 24 h at 80°C. Dried sample (50 mg) was crushed in 2.5 ml of 2.5 N HCl and then kept in boiling water bath for 3 hours. After 3 h sodium carbonate was added to it till effervescence ceases. Final volume was made 25 ml with distilled water. Again it was centrifuged and 4 ml of anthrone reagent (200 mg anthrone in 100 ml HCl) was added to 5 ml of sample and placed in water bath for 8 min. It was then cooled and optical density was recorded at 630 nm. Calibration curve of glucose was used for estimation of carbohydrate in mg g^{-1} dry weight (Fig. no - 1).

Nitrogen

Nitrogen content in leaves was determined according to the method developed by Snell and Snell (1954). Dried plant sample (50 mg) was digested in 2 ml of H_2O_2 (30%) and 5 ml of conc. solution and placed on hot plate for 30 minutes. Again, 3 ml of H_2O_2 was added and kept on hot plate for another 60 minutes or till the digest became clear. After cooling, 1 ml of digest was taken out in a test tube and 3 ml of Nesseler's reagent and 1 ml of distilled water was added to this. Optical density of coloured solution were recorded with the help of spectrophotometer at 425 nm. Total amount of nitrogen was calculated by preparing a calibration curve of ammonium sulphate and expressed as mg N_2 g^{-1} dry weight (Fig. 1).

Phosphorus

Phosphorus content was estimated according to Olsen's method (1954). Fifty mg dry material (leaves) was homogenized in 10 ml of $NaHCO_3$ (4.2 g/l) and a pinch of activated charcoal was added to this solution. It was kept on a shaker for 30 minutes and then centrifuged at 2000 rpm for 10 minutes. In a test tube 5 ml of filtrate and 5 ml molybdate reagent (15 g ammonium molybdate + 30 ml distilled water + 348 ml conc HCl + add distilled water to make 1 litre) were added and swirled. After that, 1 ml of working $SnCl_2$ solution (prepared by mixing 1 ml stock $SnCl_2$ solution (40%) and 60 ml distilled water) was added to reaction mixture. Final

volume was made upto 25 ml. Optical density of each sample was recorded at 660 nm. Calibration curve (Fig. 1) prepared by potassium hydrogen phosphate (KH_2PO_4) was used to calculate the amount of phosphorus in mg g^{-1} dry weight.

Calcium, potassium and sodium

These nutrients were estimated according to United State Pharmacopoeia (USP, 2000).

The flame photometer characteristically was equipped with a photomultiplier phototube detector (determination of calcium or sodium) a red-sensitive phototube detector (determination of potassium), a monochromator, an adjustable exit slit (sensitivity control) and an oxyacetylene burner.

Standard calcium ion solution : 249.7 mg of calcium carbonate (previously dried at 300°C for 3 h and cooled in a desiccator for 2 h) was transferred to a 100 ml volumetric flask containing a mixture of 20 ml of water. In this 5 ml of 3N hydrochloric acid was dissolved and then diluted with water to volume and mixed. Each ml of this solution contains 1.00 mg of calcium ion (Ca^+).

Standard potassium ion solution : 190.7 mg of potassium chloride (previously dried at 150°C for 2 h) was transferred to a 100 ml water filled volumetric flask and then mixed. Each ml of the solution contains 1.00 mg of potassium ion (K^+).

Standard sodium ion solution: 254.2 mg of sodium chloride (previously dried at 150°C for 2 h) was transferred into a 100 ml volumetric flask and dissolve in water to same volume. Each ml of the solution contains 1.00 mg of sodium ion (Na^+).

Standard test preparation: 50 ml aliquot of the test preparation was transferred to a 100 ml volumetric flask, add to volume of standard 10 ml ion solution, diluted with 40 ml water and mixed well. Quantitatively dilute aliquots of this solution with water as necessary to bring the concentration of the ion determined at the 630 nm wavelength for the flame photometer used.

Test preparation—Unless otherwise directed in the individual monograph, 2.000 g of test specimen was transferred to a 100 ml volumetric flask, chill in an ice bath, add 5 ml of nitric acid and swirl to obtain a clear or just slightly turbid mixture. It was cool at room temperature and diluted with water to volume and mix. After this filtered the solution to obtain a clear solution.

Dilute aliquots of the test prepare with water as a solution in which the concentration was similar to the Standard Preparation. Without changing any of the adjustments of the flame photometer, emission of the

solution was determine as percent transmittance and record the reading, labeled it as test (T). Readjust only the monochromator to the designated wavelength for background determination. Determine the emission of the solution at this wavelength as percent transmittance and record the reading, labeled it as blank (B).

The requirements of the test are met if the value of test - blank sample (T-B) is less than or equal to the value of S -T.

	Wavelength (nm)		
Ion	**Characteristic**	**Background**	**Bandwidth (nm)**
Calcium	422.7	430	0.8
Potassium	766.5	750	12
Sodium	589	580	0.8

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RESULT

Protein and carbohydrate content were also calculated in the seeds and seedlings of the all five test seedlings plants. Result of protein analysis is shown in Tables 7-12. Amount of protein in seeds of wheat, rice, maize, barley oat and rice was recorded 45.000, 25.002, 38.005, 30.908 and 32.000 mg g^{-1} f.wt., respectively. On 3rd day of seedling stage protein was amounted to 80.762, 31.921, 65.452, 54.469 and 42.876 mg g^{-1} f.wt., in wheat, maize, barley, oat and rice, respectively. The value of this biochemical component was highest 80.762 mg g^{-1} f.wt. in wheat and lowest in maize 31.921 mg g^{-1} f.wt.

Again, the analysis of biochemical component of protein in 5 day old seedlings of plants viz. wheat, maize, barley, oat and rice was 67.027, 20.416, 52.736, 48.192 and 32.169 mg g^{-1} f.wt., respectively. The value of this biochemical component at this stage was highest 67.027 mg g^{-1} f.wt. in wheat and lowest 20.416 mg g^{-1} f.wt. in maize. On 7th day seedling of wheat, maize, barley, oat and rice protein contents were 40.361, 12.032, 35.642, 30.518 and 20.687 mg g^{-1} f.wt., respectively. The value of this biochemical component was highest 40.361 mg g^{-1} f.wt. in wheat and lowest in maize 12.032 mg g^{-1} f.wt. Amount of biochemical named protein in 10 day seedlings of wheat, maize, barley, oat and rice was 38.619, 11.862, 33.969, 30.012 and 19.812 mg g^{-1} f.wt., respectively. The value of this biochemical component was highest 38.619 mg g^{-1} f.wt. in wheat and lowest 11.862 mg g^{-1} f.wt in maize. The corresponding values for protein content at 15th day was 38.196, 11.268,

33.261, 39.985 and 19.428 mg g^{-1} f.wt. (Fig. 5).

Carbohydrate contents in the seeds of wheat, rice, maize, barley and oat were amounted to 714.007, 770.234, 730.242, 370.495 and 450.201 mg g^{-1} d.wt., respectively. The values of carbohydrate in 5 day old seedlings of wheat, maize, barley, oat and rice were 84.246, 59.162, 77.024, 70.227 and 66.184 mg g^{-1} d.wt., respectively. The highest carbohydrate value was found in wheat 84.246 mg g^{-1} d.wt. and lowest 59.162 mg g^{-1} d.wt., in maize. The value of carbohydrate contents in 7 day old seedlings of wheat, maize, barley, oat and rice was 85.685, 61.233, 79.770, 71.694 and 68.301 mg g^{-1}, respectively. The highest carbohydrate value at this stage was found in wheat 85.685 mg g^{-1} d.wt and lowest in maize 61.233 mg g^{-1} d.wt. Carbohydrate content in 10 day old seedlings was observed 90.246, 59.256, 83.376, 76.193 and 63.289 mg g^{-1} d.wt., in wheat, maize, barley, oat and rice, respectively. The highest carbohydrate value on 10^{th} day was found in wheat 90.246 mg g^{-1} and lowest in maize 59.252 mg g^{-1} d.wt. The corresponding values in 15 day old seedlings were 92.114, 63.247, 85.149, 79.524 and 65.124 mg g^{-1} d.wt. On 15^{th} day the maximum amount of carbohydrate was recorded in wheat seedlings and minimum in maize seedlings (Fig. 4).

Maximum amount of lipids among seeds of five test seedlings plants was recorded in oat 35.859 mg g^{-1} f.wt. while its minimum value was amounted in rice seeds 18.032 mg g^{-1} f.wt. Further the lipid content in 3 day old seedlings of wheat, rice, maize, barley and oat was 32.446, 29.193, 54.935, 28.243 and 60.437 mg g^{-1} f.wt., respectively.

Lipid contents in 5 day old seedling plants of wheat, maize, barley, oat and rice were 31.192, 48.475, 26.243, 47.139 and 27.488 mg g^{-1} f.wt., respectively. Highest value of lipids was found in maize 48.475 mg g^{-1} f.wt. and lowest value in barley 26.243 mg g^{-1} f.wt. Analysis of lipid contents in 7 day old seedling plants of wheat, maize, barley, oat and rice showed the value 28.239, 44.359, 25.318, 43.182, and 23.021 mg g^{-1} f.wt, respectively. Highest value of lipids was found in maize (44.359 mg g^{-1} f.wt.) and lowest value in rice 23.021 mg g^{-1} f.wt. The corresponding values at 15 day old seedlings were 21.249, 20.714, 36.004, 26.169 and 28.892 mg g^{-1} f.wt in wheat, maize, barley, oat and rice (Fig. 11).

The value of α-amylase enzyme in dry seeds viz. wheat, maize, barley, oat and rice were 98.202, 60.104, 86.267, 74.641 and 65.302 mg g^{-1} f.wt , respectively. Wheat seeds showed maximum value as 98.202 mg g^{-1} f.wt. and maize show minimum value as 60.104 mg g^{-1} f.wt (Table 7).

α-Amylase enzyme observed in 3^{rd} day old seedling plants viz. wheat, maize, barley, oat and rice were 165.221, 93.487, 160.149, 15289 and 87.149 mg g^{-1} f.wt., respectively. Wheat seedling showed maximum value as 165.221 mg g^{-1} f.wt. and rice show minimum value as 87.149 mg g^{-1} f.wt., (Table 8). Again, the analysis

of α-amylase enzyme in 5 day old seedlings plant viz. wheat, maize, barley, oat and rice were 166.422, 95.126, 165.212, 161.269 and 91.732 mg g^{-1} f.wt., respectively. Amount of α-Amylase enzyme in 7 day seedlings of wheat, maize, barley, oat and rice was 168.261, 99.264, 168.043, 164.012 and 94.642 mg g^{-1} f.wt., respectively. The value of α-amylase enzyme was highest in wheat 168.261 mg g^{-1} f.wt. and lowest 94.642 mg g^{-1} f.wt. in rice. The corresponding values for α-amylase at 15^{th} day were 171.169, 103.014, 171.345, 166.007 and 98.412 mg g^{-1} f.wt. in corresponding seedlings (Table 12).

Some nutrients like nitrogen, phosphorus, calcium, iron, magnesium, potassium, zinc, sodium, copper and manganese were also amounted in the seeds and seedlings of different ages of all the five experimental crops (Tables 7-12). Amount of nitrogen in the seeds of wheat, rice, maize, barley and oat was recorded as 2.169, 1.152, 2.163, 1.324 and 1.934 mg g^{-1} f.wt, respectively. The biochemical component of nitrogen in 3 day old seedlings of wheat, maize, barley, oat and rice was 4.240, 4.627, 1.827, 1.445 and 1.824 mg g^{-1} f.wt, respectively. The highest value was found in maize 4.627 mg g^{-1} f.wt and lowest in oat 1.445 mg g^{-1} f.wt. The amount of nitrogen in 5 day old seedlings of wheat, maize, barley, oat and rice was 4.642, 4.719, 2.482, 1.627 and 2.118 mg g^{-1} f.wt, respectively. The highest values of nitrogen in 7 day old seedling of wheat, maize, barley, oat and rice were 4.749, 4.781, 2.998, 1.825 and 2.756 mg g^{-1} f.wt, respectively. The highest value was found in maize (4.781 mg g^{-1} f.wt) and lowest in oat (1.825 mg g^{-1} f.wt).

The amount of nitrogen observed in 10 day old seedlings of wheat, maize, barley, oat and rice was 5.584, 4.967, 3.062, 2.023, and 3.239 mg g^{-1} f.wt, respectively. The highest value was found in wheat 5.584 mg g^{-1} f.wt and lowest in oat 2.023 mg g^{-1} f.wt. The corresponding values for the same parameter on 15^{th} day of seedling stage were 5.781, 3.718, 5.630, 3.951 2.730 and 3.718 mg g^{-1} f.wt of tested seedlings. The maximum amount of nitrogen was found in wheat seedlings and minimum in oat seedlings on 15^{th} day (Fig. 6).

Phosphorous content amounted to 0.181, 0.064, 0.098, 0.037 and 0.047 mg g^{-1} f.wt. was noted in the seeds of wheat, rice, maize, barley and oat, respectively. The value of phosphorous in 3 day old seedlings of wheat, maize, barley, oat and rice was 1.527, 3.263, 0.637, 1.212 and 0.429 mg g^{-1} f.wt, respectively. The maize seedlings were found to contain the highest value 3.263 mg g^{-1} f.wt and lowest value 0.429 mg g^{-1} f.wt was found in rice.

The content of phosphorous in 5 day old wheat, maize, barley, oat and rice seedling was 1.002, 2.096, 0.458, 0.894 and 0.312 mg g^{-1} f.wt, respectively. The maize plants had highest value 2.096 mg g^{-1} f.wt and lowest value 0.312 mg g^{-1} f.wt in rice. The values of phosphorous in 7 day old seedlings of wheat, maize, barley, oat and rice were 0.667, 1.656, 0.303, 0.529 and 0.189 mg g^{-1} f.wt, respectively.

Phosphorous content observed in 10 day old plant seedlings viz. wheat, maize, barley, oat and rice was 0.653, 1.592, 0.296, 0.517, and 0.179 mg g^{-1} f.wt, respectively. The maize plants were found to have highest value 1.592 mg g^{-1} f.wt and lowest value was found in rice 0.179 mg g^{-1} f.wt. The minimum and maximum values of phosphorous in 15 day old seedlings were recorded in rice 0.162 mg g^{-1} f.wt and maize 1.503 mg g^{-1} f.wt while it was amounted to 0.635, 0.285 and 0.509 mg g^{-1} f.wt in wheat, barley and oat (Fig. 7), respectively.

Calcium was amounted to 2.028, 0.829, 1.802, 1.324 and 1.761 mg g^{-1} f.wt in the seeds of wheat, rice, maize, barley and oat, respectively. The value of calcium in 3 day old seedling of wheat, maize, barley, oat and rice was 0.809, 0.690, 1.942, 3.243 and 0.885 mg g^{-1} f.wt respectively. Oat plants showed highest value 3.243 mg g^{-1} f.wt and wheat lowest value 0.809 mg g^{-1} f.wt. The calcium amounted in 5 day old seedling of wheat, maize, barley, oat and rice was 0.721, 0.518, 1.426, 2.819 and 0.625 mg g^{-1} f.wt, respectively. Oat plants showed highest value 2.819 mg g^{-1} f.wt. and wheat low value 0.518 mg g^{-1} f.wt. Data on yield of calcium in 7 day seedling plants viz. wheat, maize, barley, oat and rice were 0.491, 0.511, 1.260, 2.521 and 0.449 mg g^{-1} f.wt, respectively. Oat plant showed highest value 2.521 mg g^{-1} f.wt and rice lowest value 0.449 mg g^{-1} f.wt.

Calcium content estimated in 10 days old seedlings of wheat, maize, barley, oat and rice was 0.480, 0.496, 1.186, 2.216 and 0.434 mg g^{-1} f.wt, respectively (Fig. 8). Oat plants again showed highest value 2.216 mg g^{-1} f.wt and rice with lowest value 0.434 mg g^{-1} f.wt. This results got repeated in 15 d old seedlings also (Fig. 8).

Results on iron contents in 3 day old seedlings of wheat, maize, barley, oat and rice showed 0.617, 0.339, 0.110, 0.135 and 0.138 mg g^{-1} f.wt, respectively. Wheat plants were found showing highest value 0.617 mg g^{-1} f.wt and oat with lowest value 0.146 mg g^{-1} f.wt. The values for iron contents in 5 day old seedlings of wheat, maize, barley, oat and rice were 0.704, 0.342, 0.133, 0.182 and 0.146 mg g^{-1} f.wt, respectively. Highest value was noted in wheat while lowest in oat (Table 9).

Seven day old seedlings of wheat, maize, barley, oat and rice had 0.827, 0.589, 0.216, 0.214 and 0.192 mg iron g^{-1} f.wt, respectively. Wheat plants showed highest value 0.827 mg g^{-1} f.wt and lowest value 0.192 mg g^{-1} f.wt in rice seedling plant. Iron contents in 3 day old seedlings plants viz. wheat, maize, barley, oat and rice were 1.682, 0.921, 0.282, 0.339, and 0.363 mg g^{-1} f.wt, respectively. The value of iron content in dry seeds viz. wheat, maize, barley, oat and rice were 2.903, 1.402, 1.601, 1.232 and 1.129 mg g^{-1} f.wt, respectively. Maximum amount of iron was recorded in oat 5.232 mg g^{-1} f.wt while for seedlings 15 day old, minimum value was observed in wheat 0.107 mg g^{-1} f.wt.

DISCUSSION

Proteins form the structural and functional basis of the cell. They are nitrogenous organic substances produced by and associated with living matter. Proteins occur in both the plant and animal kingdom (Abeles *et al.,* 1982). Plant proteins are easily extracted in crystalline form. Plants usually store proteins in the form of aleurone grains (Frank and Amelio Sr., 1999). Maximum amount of protein was recorded in the seeds than in the seedlings of all the five cultivars. This is due to the fact that at this stage protein along with other nutrients like carbohydrates and fats are still in a stored condition and their mobilization for germination process has still not begun.

Carbohydrates are compounds containing the elements carbon, hydrogen and oxygen. They are either aldehydic or ketonic alcohol, in which hydrogen and oxygen are present in the same ratio as in water. These are primary sources of energy in living organisms. Only after their exhaustation do the other sources of energy viz. proteins and lipids are used. As was observed for proteins, the carbohydrate content was found maximum in the seeds rather than in seedlings of the five cultivars. In the seedling stage there was a general decrease in the carbohydrate content and maximum value was observed on the final day of observation. This is probably because of the beginning and subsequent increase in the photosynthetic activity of the seedlings resulting in more carbohydrate synthesis.

Nitrogen and phosphorus actively participates in various physiological activities and any imbalance in the optima of these biochemical components ultimately disturbs the growth and yield. Rao and Kadkel (1957) during analysis of developing rice observed that on both dry and fresh weight basis total nitrogen regularly increased.

SUMMURY

SUMMURY

the present study entitled study on nutritionally important biochemicals from the seedlings of some selected plants of the family *poaceae* (wheat, rice, maize, oat and barley) the effect of amala *(phyllanthus emblica)* fruit proteins extracts was studied on selected bacterial strains for antibacterial activity at different ph and solvents. the present finding of the experiments are presented in the followings tables and figures under appropriate headings.

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