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RESEARCH PAPER

Evaluation of nutritional profile of pulses consumed in Pakistan

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Key Message: Legumes are considered as poor man's meat but they need proper recognition to utilize them in accurate amounts to overcome the required protein and amino acid in human health.

Abstract: Pulses are the world's most beneficial nourishment because of its nutritious profile. Protein quality in pulses is clearly impacted due to their chemical structure. In this study, the diverse pulses i.e. chickpea white, chickpea dark-colored, split chickpea, red lentil, yellow lentil, white lentil, red kidney bean and a black eye or goat bean were collected from the different regions of the country for comparative composition of fat solvent vitamins (A, E and β-carotene) and amino acids. The results demonstrated that pulses contained high protein contents from 18 to 24%, dietary fibre from 2 to 4%, lipids from 0.7 to 5%, dietary fiber 10.86 to 26.03%, while starch ranged from 59 to 66%. The vitamin A was found to be absent in all of variants, while vitamin E was recorded in chickpea white, chickpea dark-colored and split chickpea 1.8, 1.5 and 0.6 mg/100 g, respectively. The β-carotene was found between 8.33 to 545.00 µg/100 g in all the tested samples. The amino acids (3.4 g/100 g) were high in chickpea white and lysine was found as 4.291 g/100 g in kidney bean. Aspartic and glutamic acid contents were high in goat pea 2.667 and 5.168 g/100 g, while the arginine contents (4.33 g/100 g) were recorded in white lentil. Cysteine in yellow lentil 8.427 g/100 g and in black bean 4.543 g/100 g, respectively.

Keywords: Amino acids, Carbohydrate contents, Fat soluble vitamins, Pulses

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Introduction

Legume crops are predominant for ensuring the sustainability of agro-food systems. Among them, pulses production is subjected to strong demand compared to soybean, the leading worldwide crop. To foster more grain-legume crop diversity, scientific research is essential for providing new knowledge that may lead to new development. In legumes, starch is the most abundant carbohydrate reserve in plants and has been accredited as medicinal, cultural and nutritional roles. The major carbohydrate of kidney bean seeds is starch, which accounts for 25–45% of the dry matter. Lower swelling and high solubility of kidney bean starches indicate their higher functional properties than that of other cereal starches. High amount of resistant starch (RS), slow digestible starch (SDS) and low amount of rapidly digestible starch (RDS) in kidney bean reveal its potential as a good source of RS. Starch is a macro-constituent of many foods and its properties and interactions with other constituents, particularly water and lipids, are of interest to the food industry and for human nutrition (Punia et al., 2020).

Legumes particularly chickpeas, mung beans, soybeans, and peas have a place with the Fabaceae family and are healthfully rich food. The pulses are significant sources of plant protein in numerous weight control plans. Beans assume an important activity in metabolic and physiological procedures because of the adjacency of different bioactive compounds, and most of them are phenolic acids, flavonoids, and tannins. Legumes are additionally a trustworthy source of 15 basic minerals and nutrients, yet their bioavailability is low because of the likeness of anti-nutrient factors in it (Kumar & Pandey, 2020).

Sustainable agricultural productivity is obstructed by over-dependency on major staple crops, negligence and underutilization of other crops (legumes), change of climate, as well as land devaluation. Threats posed by these limiting factors are undoubtedly contributing to global food insecurity, increased rural poverty, and malnutrition in the less developed countries. Diverse, unwanted and exploited grain legumes are crops primarily characterized by inherent features and capabilities to withstand the effects of abiotic stress and climate change, significantly replenish the soil, as well as boost food and protein security (Popoola et al., 2019).

Legumes are indispensable for human diet in respect to their valuable and nutritive bioactive molecules. Legumes and derivative foodstuffs are rich in fibre, proteins, vitamins and some valuable phytochemicals, which exhibit important biological activities. Due to their remarkable molecular content, they are receiving great attention by researchers. Recent developments in genomics and bioinformatics led to cumulative data about legume genomes. These data could provide important information to accelerate breeding and to develop new traits for bio-fortification (Cakir et al., 2019). Grain vegetables are acknowledged for their guarantee to dietary protein and micronutrient and they offer expected advantages in developing nations where future food demand is expanding (De Jager et al., 2019)

Legumes are plants in the family Fabaceae characterized by seeds in pods that are often edible though sometimes toxic. The nutrient contents (protein, carbohydrates and micronutrients) of legumes contribute to address under-nutrition, especially protein-calorie malnutrition among children and nursing mothers in developing countries where supplementing cereal-based diets with legumes is suggested as one of the best solutions to protein calorie malnutrition. Moreover, legumes play a role in prevention, improvement and/or treatment of disease conditions such as diabetes mellitus, cardiovascular diseases, and cancer diseases e.g. breast and prostate cancers and lowers blood cholesterol level. It is, therefore, claimed that including legumes in a health-promoting diet is important in meeting the major dietary recommendations to improve the nutritional status of undernourished as well as over-nourished individuals, and to reduce risk of chronic diseases (Gebrelibanos et al., 2013).

The pulses family leguminosae consists of plants that produce a pod with seeds. Commonly edible pulses include dry beans, lentils, chickpeas, dry peas, and green beans. The term pulses or legumes used differently since all pulses are counted as legumes whereas all legumes are not pulses. The word “pulses” is described as the crops harvested solely for dry seeds of leguminous plants. Pulses have been nutritious food for many civilizations within the universe over 10,000 years. Their consumption dates back to as far as 5500 B.C. and they are said to be one of the oldest crops cultivated by mankind. They are valued around the universe as a poor man’s meat or alternative to meat (Kouris-Blazos & Belski, 2016). The nutritional attributes of pulses studied to improve physiological benefits in humans have been scrutinized widely. Pulse grain is abundant in carbohydrate, protein, fiber and rich source of other nutritional compounds (Tharanathan & Mahadevamma, 2003; Bessada et al., 2019). Pulses are a predominant source of macronutrients and produce good protein (Siddhuraju et al., 2002; Escudero et al., 2006). Regular consumption of pulses approximately 3 to 4 times in contrast with at minimum once a week has been corresponding to fight against cardiovascular diseases (Flight & Clifton, 2006). Legumes or pulses have an average twofold protein than that of other cereal grains (Vijayakumari et al., 1997; Rebello et al., 2014).

Food crops such as pulses and cereals have a conspicuous corner in the human diet as they are the main cause of energy for a wide population (Mal, 1992; Siddhuraju et al., 2000; Kalidass & Mohan, 2011). Pulses are the primary origin of proteins in human food, in addition to carbohydrate and fiber (Rochfort & Panozzo, 2007). Pulses serve a high amount of protein 17-30%, whereas cereals not only give protein supplements but also provide minerals and water-soluble vitamins (Friedman, 1996). The objective of the study was to assess the pulses available in Pakistan for their profile of fat-soluble vitamins and amino acids.

Materials and Methods

Samples collection and preparation

Samples of eight different pulses namely Chickpea white, Chickpea brown, Split chickpea, Red lentil, Yellow lentil, White lentil, Black eye bean and Kidney bean were collected from all provinces of Pakistan (Sindh, Punjab, Balochistan, and KPK) and brought to the Laboratory of Food and Marine Resources Research Center, PCSIR Laboratories Complex, Karachi, Pakistan. The samples of the same pulses from different regions were mixed and blended to a fine powder and made a composite sample of each pulses. The samples were vacuum sealed in an aluminum foil bag and stored at room temperature (25 ºC).

Proximate composition

The proximate composition of pulses including moisture, ash, fat, and protein were analyzed by (Association of Official Analytical Chemists [AOAC], 2012) and total carbohydrate was calculated by difference. The dietary fiber was analyzed by enzymatic method TDF assay megazyme (McCleary et al., 2015).

Fat soluble vitamins

Fat-soluble vitamins were determined by the HPLC method (Sami et al., 2014). 10g of each powdered sample was taken in a screw cap test tube (100 ml), and 3 ml of 50% KOH, methanol 30 ml, and pyrogallol 0.1 g were added as an antioxidant. Tubes were kept in a hot water bath at 100 ºC for saponification. They were allowed to cool and then 30 ml water was added. The whole sample was poured in a separating funnel, and diethyl ether was added, lower layers were removed, and upper layer was taken. Washing was done first with diethyl ether 4 times and then with water. Completely washed and transparent layer in a round bottom flask was collected and rotated to remove diethyl ether. Further nitrogen gas was used to dry more. 10 ml methanol was added for volume makeup, filtered through 0.45 µm and poured in a vile and subjected to HPLC analysis. Fat-soluble vitamins were classified by measuring their graphical readings with authentic standards. Standards were made by dissolving 1 mg in 100 ml methanol. Dilution of different concentrations was prepared as 0.1, 1, 2, and 5 of each vitamin A, E and β-carotene, respectively. The mobile phase used was 95% methanol and 5% water.

Amino acid analysis

A sample size of 300 mg taken with set of three from every variant, hydrolyzed with 6 M HCl in an emptied test tube for 24 h at 105 °C. The dried deposit was disintegrated in citrate support (pH 2.2) after blaze vanishing. Aliquots were examined in a programmed amino acid analyzer (Hitachi Perkin– Elmer Show KLA 3B), utilizing the cradle framework portrayed before (Khalil & Durrani, 1990). Methionine and cystine were investigated independently after performing acidic digestion and resulting hydrolysis with HCl. Tryptophan was detected after caustic (NaOH) hydrolysis by the conventional technique.

Anti-nutritional compound

Phytic acid content was analyzed by enzymatic method, total phosphorus assay megazyme (McKie & MccleAry, 2016).

Statistical analysis

The obtained data of proximate composition, fat-soluble vitamins (A, E & β-carotene) and amino acid analysis were evaluated in triplicate determination. The data was subjected to ANOVA) at $p<0.05$ as described by Steel et al. (1980) using a computer based software, Statistix 8.1.

Results

Proximate composition

The chemical composition of selected pulses samples is given in Table 1 & 2. The protein contents varied from the lowest values of 18% in white chickpea and kidney bean, followed by 19% in split chickpea, while the highest value of protein contents (23%) were found in white and yellow lentil. The ash of pulses was found in the range of 2.3% in red lentil compared with 3.9% in red kidney bean as higher levels. The moisture contents ranged from 9% in white lentil and higher in red lentil (12%). Low moisture contents of pulses gave a longer shelf life to pulses. The lipid content of pulses was also very low from 0.7% in yellow lentil to 4.7% in chickpea white. Other pulses such as kidney bean had 1.0% lipid contents. Carbohydrates were found in higher contents in all types of pulses ranged from 59.7% to 65.5%.

Table 1 Proximate composition (protein contents, ash contents and moisture contents) of pulses (g/100 g)

Samples	PC	AC	MC
Chickpea white	18.537±0.14	2.779±0.023	10.302±0.02
Chickpea brown	18.883±0.58	2.681±0.057	10.102±0.02
Split chickpea	19.772±0.46	2.662±0.307	11.384±0.03
Red lentil	19.063±0.63	2.332±0.046	12.084±0.08
Yellow lentil	23.796±0.72	2.909±0.152	12.798±0.04
White lentil	22.652±0.33	3.206±0.120	9.649±0.01
Black eyed bean	22.571±0.29	3.219±0.118	11.041±0.03
Kidney bean	18.115±0.66	3.905±0.057	11.305±0.10

PC = Protein contents (g/100 g); AC = Ash contents (g/100 g); MC = Moisture contents (g/100 g); All the values are means of triplicate determination on dry basis ± denotes standard deviation; The statistical analysis shows significant differences ($P<0.05$) between mean values of all pulses.

Table 2 Proximate composition (Fat contents, carbohydrate contents and dietary fiber contents) (g/100 g) of pulses

Samples	FC	CC	DFC
Chickpea white	4.846±0.26	63.535±0.13	24.77± 0.10
Chickpea brown	4.295±0.24	64.127±0.48	26.033±0.08
Split chickpea	4.031±0.07	62.151±0.59	13.470±0.07
Red lentil	0.815±0.02	65.570±0.69	10.868±0.12
Yellow lentil	0.746±0.13	59.750±0.77	15.905±0.65
White lentil	1.044±0.39	63.351±0.35	17.432±0.14
Black eyed bean	1.005±0.09	62.163±0.33	17.643±0.11
Kidney bean	1.391±0.15	65.384±0.55	22.622±0.37

FC = Fat contents (g/100 g); CC = Carbohydrate contents (g/100 g); DFC = Dietary fiber contents (g/100 g); All the values are means of triplicate determination on dry basis ± denotes standard deviation; The statistical analysis shows significant differences ($P<0.05$) between mean values of all pulses.

Vitamins

The results of fat soluble vitamins are given in Table 3. The fat soluble vitamins (including vitamin A) were absent in all the samples of pulses. Vitamin E was found in chickpea white (1.8 mg/100 g), chickpea brown (1.5 mg/100 g) and split chickpea (0.6 mg/100 g). While the β-carotene was found in all samples as low as 8.3 µg/100 g in black eye bean and as high as 545 µg/100g in white lentil.

Table 3 Fat soluble vitamins (µg/100g) of pulses

Samples	β-carotene (µg/100 g)
Chickpea white	196.33±2.52
Chickpea brown	336.33±5.51
Split chickpea	170.00±2.00
Red lentil	16.33±0.57
Yellow lentil	133.33±3.21
White lentil	545.00±2.00
Black eyed bean	8.333±0.55
Kidney bean	19.667±0.55

All the values are means of triplicate determination on dry basis ± denotes standard deviation. The statistical analysis shows significant differences ($P<0.05$) between mean values of all pulses.

Amino acids

The results of non-essential and essential amino acids (A.As) are given in Table 4 & 5, respectively. The obtained results of the study conclude that pulses contained high percentages of both essential and non-essential A.As. Essential A.As leucine, isoleucine, phenylalanine and histidine were found to be the highest in chickpea white which were 3.400, 0.507, 1.836 and 0.134 g/100 g, respectively, while valine in goat pea was found 0.560 and lysine was found 4.291 g/100 g in kidney beans. Non-essential A.As aspartic, glutamic acid, glycine, alanine was the most noteworthy in black eyed bean which were 2.677, 5.168, 1.236 and 1.631 g/100 g individually, while the arginine was found 4.330 g/100g in white lentil. Sulphur containing A.A cysteine was found to be in astounding amount in yellow lentil (8.427 g/100 g) and in black eyed bean (4.543 g/100 g) and methionine in split chickpea was found to be as 0.799 g/100 g. The results of phytic acid are given in Table 6 Phytic acid was very low in all variants; very minimum 0.004 g/100 g to the highest 1.01 g/100 g in chickpea white and brown, respectively.

Table 4 Non-essential amino acids contents (g/100 g) of pulses

Samples	Aspartic acid	Serine	Glutamic acid	Proline	Glycine
Chickpea white	1.980±0.01	0.190±0.00	3.534±0.03	0.509±0.01	0.914±0.09
Chickpea brown	2.235±0.00	0.177±0.02	4.214±0.09	0.993±0.01	1.008±0.01
Split chickpea	2.440±0.00	0.442±0.02	4.565±0.02	1.060±0.06	1.042±0.02
Red lentil	2.114±0.00	0.117±0.00	4.081±0.19	1.565±0.11	0.889±0.00
Yellow lentil	1.960±0.00	0.360±0.01	4.048±0.02	0.822±0.03	0.778±0.01
White lentil	2.231±0.01	0.213±0.00	4.291±0.06	0.140±0.04	1.062±0.05
Black eye bean	2.677±0.01	0.237±0.01	5.168±0.03	1.037±0.03	1.236±0.02
Kidney bean	1.637±0.02	0.227±0.00	2.610±0.04	0.891±0.10	0.713±0.01

All the values are means of triplicate determination on dry basis ± denotes standard deviation. The statistical analysis shows significant differences (P<0.05) between mean values of all pulses.

Table 4 continue

Samples	Alanine	Cysteine	Tyrosine	Arginine
Chickpea white	1.193±0.02	1.177±0.02	1.959±0.06	0.000±0.00
Chickpea brown	1.263±0.03	1.994±0.11	0.665±0.00	0.000±0.00
Split chickpea	1.301±0.00	1.150±0.06	0.628±0.05	2.503±0.05
Red lentil	1.396±0.01	1.665±0.06	0.672±0.02	2.878±0.00
Yellow lentil	1.061±0.03	8.427±0.07	0.494±0.02	0.979±0.01
White lentil	1.336±0.05	0.561±0.00	0.808±0.00	4.330±0.05
Black eye bean	1.631±0.08	4.543±0.02	0.615±0.00	0.000±0.00
Kidney bean	0.854±0.05	0.344±0.01	0.513±0.01	3.015±0.10

All the values are means of triplicate determination on dry basis ± denotes standard deviation. The statistical analysis shows significant differences (P<0.05) between mean values of all pulses.

Table 5 Essential amino acids contents (g/100 g) of pulses

Samples	Threonine	Valine	Methionine	Isoleucine	Leucine
Chickpea white	0.000±0.00	0.408±0.00	0.672±0.09	0.507±0.01	3.400±0.01
Chickpea brown	0.000±0.00	0.445±0.00	0.515±0.09	0.053±0.00	1.655±0.08
Split chickpea	0.083±0.00	0.327±0.03	0.799±0.00	0.008±0.00	1.419±0.00
Red lentil	0.074±0.00	0.476±0.02	0.325±0.01	0.019±0.00	1.509±0.10
Yellow lentil	0.042±0.00	0.304±0.00	0.385±0.01	0.018±0.00	1.323±0.06
White lentil	0.000±0.00	0.496±0.02	0.258±0.03	0.084±0.01	1.885±0.07
Black eye bean	0.000±0.00	0.560±0.03	0.745±0.06	0.026±0.00	1.762±0.01
Kidney bean	0.000±0.00	0.279±0.02	0.336±0.00	0.116±0.00	0.918±0.00

All the values are means of triplicate determination on dry basis ± denotes standard deviation. The statistical analysis shows significant differences (P<0.05) between mean values of all pulses.

Table 5 continue

Samples	Phenylalanine	Histidine	Tryptophan	Lysine
Chickpea white	1.836±0.04	0.134±0.00	0.000±0.00	0.000±0.00
Chickpea brown	1.135±0.04	0.000±0.00	0.297±0.01	2.476±0.01
Split chickpea	0.991±0.00	0.011±0.00	0.333±0.00	0.759±0.03
Red lentil	0.940±0.01	0.000±0.00	0.316±0.00	0.548±0.01
Yellow lentil	1.006±0.00	0.018±0.00	0.323±0.01	1.551±0.05
White lentil	1.207±0.01	0.084±0.01	0.364±0.00	2.557±0.38
Black eye bean	1.064±0.03	0.000±0.00	0.000±0.00	0.660±0.02
Kidney bean	0.755±0.02	0.110±0.08	0.328±0.01	4.291±0.01

All the values are means of triplicate determination on dry basis ± denotes standard deviation. The statistical analysis shows significant differences (P<0.05) between mean values of all pulses.

Table 6 Phytic acid contents (µg/100g) of pulses

Samples	Phytic acid contents
Chickpea white	0.044±0.00
Chickpea brown	1.015±0.00
Split chickpea	0.617±0.00
Red lentil	0.974±0.01
Yellow lentil	0.473±0.01
White lentil	0.777±0.00
Black eyed bean	0.105±0.00
Kidney bean	0.356±0.00

All the values are means of triplicate determination on dry basis ± denotes standard deviation. The statistical analysis shows significant differences (P<0.05) between mean values of all pulses.

Discussion

The protein content of legumes cultivars grown in Pakistan ranges between 22.89% and 24.82% (Iqbal et al., 2006). According to the study of Wang et al. (2009), total mineral contents of lentils ranged between 2.50 to 2.80%. Similar data for lipid contents were also reported by Alajaji & El-adawy (2006) for local Egyptian chickpea. The pulses are rich in carbohydrates which are an important constituent to retain the patients' gut in the fine conditions and provide energy (Shad et al., 2009). The carbohydrate contents of pulses are reported to be high in the previous research study by Reddy et al. (1985); Oke et al. (1995) who also reported that the dietary fibre amount of pulses was 10.86% to 26.03%. Vitamin A refers to a large group of nutritionally active retinoids and certain carotenoids possessing the biological activity of retinol (Blomhoff, 2006). Vitamin E is an essential fat soluble vitamin for humans (Nordic Council of Ministers [NCM], 2014). The best known are β-carotene which exhibits the greatest pro-vitamin A activity (Damodaran et al., 2008). Previous analysis of adult students (≥18 years) enrolled in the National Health and Nutrition Examination Survey (NHANES) found that those who consume chickpea and other legumes in their daily diet have higher intakes of vitamin A (2623.33 ± 140 International Units/day), vitamin E (0.01 ± 0.0005 mg/day) (O'Neil et al., 2014).

Pulses contain higher quantities and varied range of A.As than any other plant species which is consumed as human diet (D'Mello, 1991). It is recognized that amino acid profile of proteins in pulses are uneven. By their comparison with other protein, the essential A.As are at lower concentration (Mahe et al., 1994). In a previous research study by Pirman et al. (2001), French green lentil was used as a sample material. Investigated parameters were reported as protein contents 26.7% from which 3.64% for isoleucine, 6.57% for leucine, 0.59 for methionine, 4.67% for phenylalanine, 3.33% for threonine, 4.02% for valine, 3.65% for alanine, 6.36% for arginine, 0.07% for cysteine and 2.09% for histidine. Another previous research in which a total of 35 new lentil varieties exhibited the following values (%); 25.329 for protein, 0.34-0.56 for isoleucine, 0.68-0.98 for leucine, 0.09-0.2 for methionine, 0.49-0.82 for phenylalanine, 0.41-0.79 for threonine, 0.06-0.09 for tryptophan, 0.53-0.90 for valine, 0.66-0.10 for arginine, 0.36-0.61 for histidine (Alghamdi et al., 2014). Pulses have a good proportion of A.As. The limiting of the A.AS are the cysteine and methionine which are sulphur containing amino acids as it observed on most of the legume seeds. Pulses have shown sufficient to high levels of lysine (Shewry & Halford, 2002). Content of A.A in food sources and nutrition are predominant topics in biological sciences. A.As show a wide array of chemistry, physiology, metabolism, immunology, pathology, reproduction and cell biology. These are fundamentals forerunners for the combination of hormone and low atomic weight nitrogenous mixes, with each having huge natural potential (Kharitonov et al., 1995; Friedman & Levin, 2012; Lei et al., 2012; Fernstrom, 2013). Amino acids improve the important metabolic pathways that are necessary for provision, prosperity, development, reproduction, lactation, and immunity. Phytate has an effect of reducing the absorbance of nutrients, due to its capability to decrease the biological availability of essential minerals and to form a complex with proteins, results in suppressing the enzymatic metabolism of consumed protein (Vijayakumari et al., 1996). Similar findings of phytic acid content of evaluated legume samples were found, contrasted with that of some generally utilized legumes *Vigna mungo* (Kataria et al., 1989), *Dolichos lablab* var. vulgaris (Vijayakumari et al., 1995), tribal pulses *Mucuna pruriens* var, utilis (Janardhanan et al., 2003) and *Mucuna atropurpurea* (Kala & Mohan, 2010).

Conclusion

It is inferred that examined pulses are incredible protein sources to diminish the issue of protein inadequacy and battle the ailing health factor. The results of this study exhibit the significantly sustaining assessment of legumes and the work in sound subsistence. Pulses are the low-cost source of protein, carbohydrates, vitamins, fiber, carotene, and health-promoting amino acids. From the above-investigated samples split chickpea and yellow lentil were found to be highly nutritious pulses in all evaluated parameters especially in amino acids.

Authors Contributions: O.M.T. conceived the idea. M.S.H. supervised the research. H.S. executed the research and A.H.S. edited the manuscript.

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