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Physico-Chemical Analysis and Functional Assessment of different cultivars of Barley (*Hordeum vulgare* L.)

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Abstract

This study employed Principal Component Analysis (PCA) to investigate the chemical and nutritional properties of two barley cultivars, PL-751 and PL-830. Using the Kaiser criterion, the first three principal components (PCs) with eigenvalues greater than one (4.23, 10.57, and 1.64) were extracted. These components accounted for more than 100% of the variance, indicating potential cumulative rounding errors or overlapping contributions. PC1 explained 88.0% of the variance for PL-751 and 82.8% for PL-830, with significant influence from color parameters (L, a, b) in PL-751 and crude protein in PL-830. PC2 was dominated by ash content in PL-751 and a mix of protein, fiber, and color parameters in PL-830. PC3 highlighted crude fat and fiber in PL-751 and dimensional properties in PL-830. The PCA biplot showed that samples associated with carbohydrates clustered on the positive side of PC1, while proximate components were significant differentiators. These findings are consistent with previous studies on barley's chemical composition. The study concludes that proximate components are key variables for distinguishing between barley cultivars, and further analysis is needed to address the overlapping contributions in the variance explanation.

Keywords: *Barley, Starch, protein, physicochemical parameters, β-glucan, functional foods*

1. Introduction

Barley, scientifically known as *Hordeum Vulgare*, is the grass that belongs to the family Poaceae and the genus *Hordeum*. It ranks fourth worldwide after wheat, rice and corn. Barley is a versatile and adaptable crop that can tolerate abiotic stress conditions. Barley shows very good resistance to dry heat compared to other grains such as wheat, rice and maize. In 2003, the world barley production was 132 million tons [1]. In 2017, barley production increased to 147 million tons from 47 hectares [2]. The annual barley production was 144 million tons in 2014, and the major barley producers are Canada, Russia, France, Germany, Australia, Ukraine and India. Canada is the largest barley producer, with a production of 13.5 million tons, holding about 10.2% of total world production. The barley production was around 150 million tons during 1970-2000, and little increase was seen during the 1980s. But a decrease in production was seen during 2000-2006 when the annual production reduced to 140 million tons. [3]. In India, barley is cultivated in nearly 7.604 lakh hectares, with a total production of 13.30 million metric tons and a productivity of 1,888 kg/ha**.** In 2019, annual barley production in India was 402,966 tons, reduced to 161,802 tons in 2020 [4].

Barley contains nearly 70-75% starch, 10-20% protein, 2-3% free lipids, 2.5-3% minerals, 11-34% dietary fibres and 5-10% β-glucan, depending upon the type of barley cultivar. [5].

Barley is rich in water-soluble and insoluble vitamins, such as vitamins B1, B2, B3, and E. It is the only cereal rich in tocols, mainly tocopherols and tocotrienols [6]. Barley is commercially used in producing beer and poultry feeds but is limited in its use in human food applications. However, it is extensively utilized in the food industry as a cheap source of starch and starch derivatives.

Barley is considered a good source of soluble and insoluble dietary fibre, particularly β-glucan, a 1-3, 1-4-b-D-glucan polysaccharide. Barley and oats have a high beta-glucan content, while other cereals contain nearly 1% beta-glucan in wheat. Beta-glucan content in oats is 3-7%, and in barley, it is 5-11% [7]. β-glucan is a soluble dietary fibre, which is a water-soluble polysaccharide. It is a glucopyranosyl unit in which about 70% is linked through 1’4 linkages and 30% through 1’3 linkage. β-glucan makes barley a functional food as it is rich in phytochemicals and antioxidants and helps lower blood cholesterol, glycaemic index, and risk of coronary heart disease [8][9]. Barley is becoming popular nowadays due to the presence of β-glucan. β-glucan acts as a thickening agent and hydrocolloid, showing its great pharmaceutical usage[10]. Barley is used to prepare pasta, bread, baby foods, soup, porridge, rice extender, breakfast cereals, etc.

Barley is traditionally a cereal choice for malting and brewing purposes due to the numerous amounts and types of enzymes produced during fermentation. [11]. Barley can survive under harsh winter conditions, is resistant to drought, plant matures early and is economical to cultivate [12]. Incorporating barley in the human daily diet is reported to reduce various health disorders such as high blood pressure, gallstones, chronic heart disease, and cancer, enhance the immune system, and help maintain a healthy colon [13, 14]. Barley contains various bioactive compounds, such as minerals, fibre, vitamins, and several phytochemicals, such as flavonoids, sterols, lignans, phenolic compounds, folates, and vitamin E [15]. Phenolic compounds show functionality in reproduction, growth and defence mechanisms against parasites, pathogens, etc. Tocols and sterols protect against toxins and neurological disorders such as Alzheimer's and diabetes. Barley overcomes the drawbacks of other major cereals such as wheat, rice, oats and rice in terms of phytochemical diversity [16, 17].

The major component of barley kernel is starch, which comprises up to 70% dry weight [18]. Due to its abundance, cheapness, biodegradable and non-toxic behaviour, food and non-food industries showgreat interest in starch usage [19]. Commonly, barley contains nearly 25-30% amylose and 70-75% amylopectin ratio, and amylose is chained through α-1, 4 glycosidic linkage and amylopectin is chained through α-1,6 glycosidic linkage. Amylose is linearly structured and has a long chain of glucosyl units that may be up to thousands of units, whereas amylopectin is heavily branched and has short chains of glucosyl units [20]. Due to the increasing acceptability of starch in food, alternative sources of starch that are cheap, affordable, and have good functional properties are being explored. Therefore, barley, an unexploited food crop, is a cheap alternative starch source.

Protein plays an important role in the human diet. It helps fulfil the body's needs by carrying out complex metabolic changes, i.e., the synthesis and breakdown of the body's protein [21]. The functions and properties of proteins are determined by their structure. Proteins can form and stabilize emulsions and enhance solubility through water and protein interactions. Proteins also show hydrodynamic properties such as gelation moulding sensory properties such as taste, smell and texture, based on intermolecular interactions.

The functional properties of molecule depend on size, shape, flexibility, Hydrophobicity, Hydrophobicity, susceptibility to denaturation, amino acid composition, charge distributions of molecules, environmental conditions (such as pH, temperature, ionic strength and pressure) and relation of protein to other food components (22). Unfortunately, only 2% of the total world production of barley is utilized for human consumption due to the presence of

husk, which is difficult to remove and cannot be used in leavened bakery products due to the absence of gluten protein. [23]. Barley also contains a significant amount of polyphenol oxidase, which reacts with phenolic compounds or amino acids that cause discoloration of food products made from barley, thus limiting the incorporation of barley in food. [24].

2. Materials and Methods

The two barley cultivars, Pl-751 and PL-830, used in the current research were procured from Eternal University, Baru Sahib, Distt Sirmaur, HP. The chemicals used in the current research were bought from standard companies such as Hi-Media, SRL, and Sigma. The physiochemical assessment of two varieties of barley cultivars, i.e., PL-751 and PL-830, was done at the laboratories of Eternal University, Baru Sahib, Distt Sirmaur, HP, India. The moisture content was determined using the air oven drying method described in [25]. The crude protein content was estimated using the Kjeloplus system (Pelican Inc.), crude fibre by the Fibroplus system (Pelican Inc.) and crude fat was determined using the Soxoplus system (Pelican Inc.) and the ash content was measured as per the method described by [26]. The total carbohydrates were calculated by subtracting the combined moisture, protein, fat, fibre, and ash content from 100. β-glucan content was analyzed as per the method given by [27]. The colour of all samples in the present study was measured using a chromameter (CR-400, Konica Minolta, Japan) at Eternal University, Baru Sahib. The measurement was recorded in terms of L, a, and b values, where L indicates lightness (ranging from 0 to 100, with 0 being black and 100 being white). The ‘a’ value denotes the colour spectrum from green (-) to red (+), and the ‘b’ values represent the spectrum from blue (-) to yellow (+). 1000 kernel weight was measured according to the method given by [28]. Bulk density was estimated by the method described by [29], and tap density by the methodology described by [30]. Vernier Calliper evaluated the average size of the kernel, i.e., length, width, and height of barley kernels.

2.1. Mineral Content

Mineral profiling of two cultivars of barley, i.e., PL-751 and PL-830, was carried out using ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) (PerkinElmer, USA) at Eternal University, Baru Sahib.

2.2. Statistical Analysis

Data analysis was performed using Origin Pro 2022 software, doing the PCA analysis.

3.0. Results and Discussion

3.1. Physico-chemical characteristics of different cultivars of barley

Using the Kaiser criterion, which involves selecting principal components (PCs) with eigenvalues greater than one, the first three PCs were extracted, with eigenvalues of 4.23, 10.57, and 1.64, respectively. Interestingly, these three components collectively accounted for more than 100% of the variation in the analyzed samples, likely due to cumulative rounding errors or overlapping contributions from the components, which warrants further scrutiny for accurate interpretation.

Table 1 Principal Component analysis and loading of first three components of barley cultivars

	PL-751			PL-830		
Factor Number	PC 1	PC 2	PC3	PC 1	PC 2	PC3
Initial Eigenvalues	4.23	0.57	0.000	1.64	0.34	000
% of variance	0.88	0.12	000	0.82	0.17	0.000
Cumulative %	88.0	100	100	82.8	100	100
Factor Loadings						
Moisture content	**0.359**	0.167	-0.171	-0.045	0.006	**-0.063**
Crude protein	**0.073**	-0.033	0.052	**0.045**	-0.006	-0.019
Crude fat	0.073	-0.033	**0.214**	0.004	0.171	-0.024
Crude fibre	0.081	-0.162	**-0.437**	-0.035	**-0.038**	-0.110
Ash content	0.010	**0.071**	0.071	-0.049	**-0.165**	-0.124
Carbohydrates	**-0.597**	-0.011	-0.299	0.080	0.033	**0.209**
Beta glucan content	0.026	-0.048	**-0.143**	0.009	**0.112**	-0.002
L	**0.650**	0.189	-0.201	0.732	**-0.093**	0.027
A	**0.084**	0.366	0.208	-0.269	0.034	**-0.248**
B	-0.005	0.038	**0.068**	0.258	**-0.478**	0.187
1000 kernel weight	0.039	0.265	-0.002	0.210	0.033	**0.344**
Bulk density	-0.179	**0.584**	-0.022	0.449	-0.057	**-0.480**
Tap density	-0.179	**0.584**	-0.022	-0.247	**-0.824**	-0.037
Length	0.018	-0.058	**0.626**	-0.045	0.006	**-0.034**
Width	0.010	0.071	**-0.368**	0.045	-0.006	**-0.689**
Height	-0.018	**0.058**	0.000	-0.025	**-0.082**	-0.000

For the barley cultivars PL-751 and PL-830, the first three PCs explained a significant proportion of the variance. For PL-751, PC1 accounted for 88.0% of the variance, PC2 for 100%, and PC3 for 100%. Similarly, for PL-830, PC1 explained 82.8% of the variance, PC2

100%, and PC3 100%. This indicates that PC1 holds substantial importance, particularly influenced by colour parameters (L, a, b) in PL-751 and crude protein in PL-830. The second and third PCs further elucidate the remaining variance, with PC2 in PL-751 dominated by ash content and in PL-830 by a combination of protein, fibre, and colour parameters.

The third component is influenced by crude fat and crude fiber in PL-751 and by dimensional properties in PL-830, highlighting the significance of proximate components in differentiating the samples. Figure 1 reveals that samples on the positive side of PC1 are associated with higher carbohydrate content, while those on the negative side relate to other proximate components, emphasizing the role of these components in distinguishing barley samples. Previous studies have reported similar ranges for moisture, protein, carbohydrate, crude protein, crude fat, crude fiber, ash content, and β-glucan in barley, aligning with the current findings.

Similar results were reported by (33) and reported that the Principal Component Analysis (PCA) was used to assess six barley cultivars at tillering, jointing, and booting stages. Deng et al., (33) studied the PCA of Barley cultivars and reported that the first PCA axis (43.31% variance) correlated with phytochemical constituents, and the second axis (42.65% variance) with antioxidant potential. The distinct separation of developmental stages, indicating that tillering and booting stages have higher chlorophyll, carotenoid, and protein content, and enzyme activities, while, jointing stage has higher SOD activity, highlighting the developmental stage's impact on barley quality (22).

Principal Component Analysis (PCA) classifies sample groups based on chemical composition without prior knowledge of the sample categories. In this study, PCA clearly separated the three developmental stages of barley grasses along PC1 and PC2, indicating distinguishable compounds. Changes in antioxidant enzyme activities across stages are linked to chemical transformations and enzyme interactions (24).

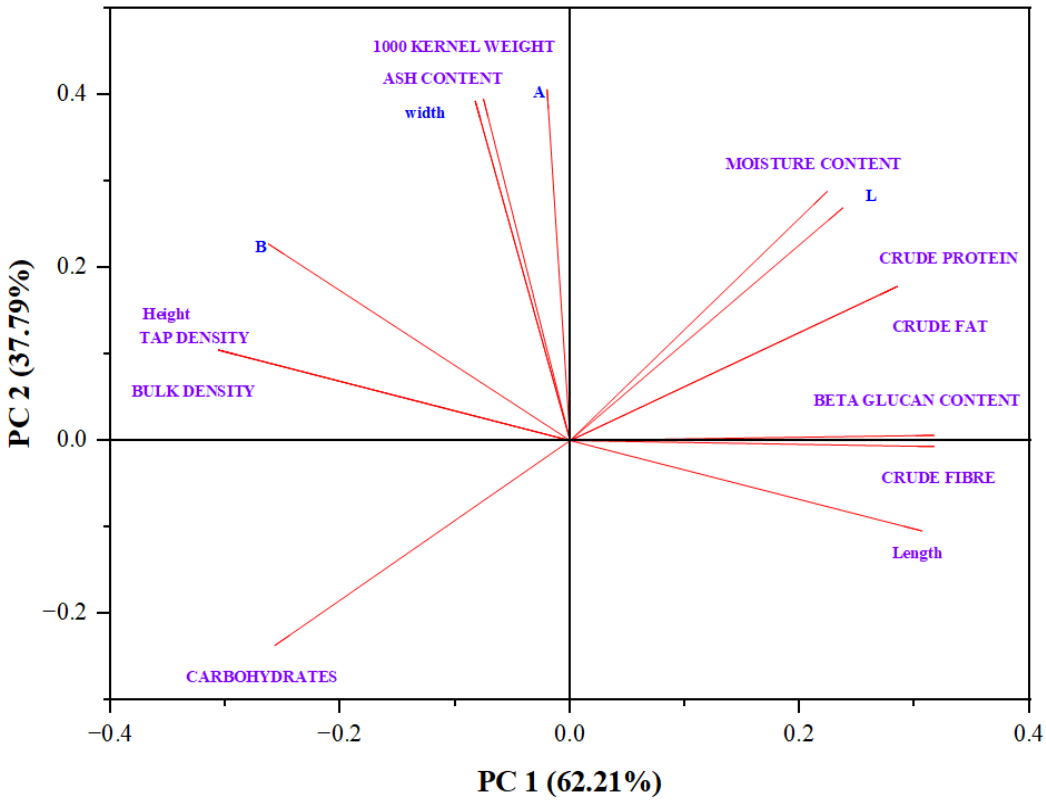

Figure. 1. Projections of the variables on the factor plane PL-751.

3. Conclusion

Barley is also a significant crop other than wheat and rice. Barley is a functional food rich in bioactive compounds such as β-glucan and antioxidants. When incorporated with other food products, Barley may help create value-added products. Therefore, barley should be promoted more for human consumption. Incorporation of barley in the daily human diet helps reduce the risk of hypertension, gallstones, cardiovascular disease, and cancer, enhances the immune system, and helps maintain a healthy colon. Barley is widely cultivated and is readily available in the market, thus making it a cheap and economical source for starch and protein extraction. he PCA results underscore the importance of proximate components in differentiating barley cultivars, consistent with prior research. Further investigation into the overlapping contributions in the explained variances could enhance the robustness of PCA interpretation in barley analysis.

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