



HAL
open science

Chemometric characterization of twenty barley varieties/lines based upon mineral profiling

Nida Kanwal, Aamir Shehzad, Rizwan Shukat, Anjum Zia, Imran Pasha

► **To cite this version:**

Nida Kanwal, Aamir Shehzad, Rizwan Shukat, Anjum Zia, Imran Pasha. Chemometric characterization of twenty barley varieties/lines based upon mineral profiling. *Pakistan Journal of Agricultural Sciences*, 2023, 60 (02), pp.273-282. <10.21162/PAKJAS/23.490>. <hal-04403964>

HAL Id: hal-04403964

<https://hal.science/hal-04403964v1>

Submitted on 27 May 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY 4.0 - Attribution - International License

Chemometric characterization of twenty barley varieties/lines based upon mineral profiling

Nida Kanwal^{1,2*}, Imran Pasha¹, Aamir Shehzad³, Rizwan Shukat¹ and Anjum Zia⁴

¹National Institute of Food Science and Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture, Faisalabad, Pakistan; ²Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, USA; ³Food Science & Agro Industry, UniLaSalle International Campus de Rouen-Normandie Universite, Mont St Aignan Cedex, France; ⁴Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan

*Corresponding author's e-mail: nidakanwal26@gmail.com

Different varieties of cereal grains differ in their nutritional composition and product making characteristics. Barley is an underutilized and neglected food crop in Pakistan. Mainly barley is used as source of carbohydrates but is also an imperative source of minerals specially potassium, calcium and phosphorus. Although, the importance of these minor constituents as minerals is of significant importance for human nutrition, the micronutrients of different Pakistani barley varieties have not been studied up till now. The aim of this study was to characterize the mineral profile of Pakistani grown barley varieties from different geographical regions. Ash content in different barley varieties ranged between 1.32-2.00%. Potassium (409-585 mg/100g) and phosphorus (307-446 mg/100g) were found to be highest minerals in all varieties while selenium (0.04-0.30 mg/kg) and cobalt (3.1-24.2 µg/kg) were found in trace amount. Overall, the varieties grown in Khyber Pakhtoonkhwa (KPK) province were found to be potentially good source of minerals especially iron and calcium. However, the varieties grown in Sindh province were found to be poor sources of minerals excluding sodium. Three statistically significant clusters were identified using chemometric tools. Barley variety Rakhshan-10 showed highest ash content and overall presented better mineral profile. Significant differences in mineral content were found in all barley varieties grown in different locations. Overall, findings of this study will help the food industries in selection of barley variety/ies with high mineral profile to be incorporated in barley-based food products.

Keywords: Cereals, macro and micro minerals, varietal differences, ash content, ICP-OES.

INTRODUCTION

Barley occupies 4th position among major cereal crops after wheat, maize, and rice worldwide, with total production 141 Mt and harvested area 47 M ha (FAO, 2018). Mainly barley is used as a source of carbohydrates but is also an important source of dietary fiber, fat, protein, and minerals specially calcium and phosphorus (Baik and Ullrich, 2008). According to Sullivan *et al.* (2013) ash concentration in barley ranges from 2 to 3% with the maximum concentration of minerals in embryo and lowest in the endosperm. Magnesium (Mg), phosphorus (P), potassium (K), and calcium (Ca) were reported to be major minerals in barley with phosphorus and potassium being the highest ones (Chappell *et al.*, 2017). Minerals are inorganic elements and an important constituent in the daily diet of human beings and are divided into two groups: (i) macro and (ii) microminerals. Macrominerals comprise of those that are required in large amounts such as

calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) while micro minerals are needed in small quantity such as copper (Cu), zinc (Zn), iron (Fe), boron (B) and selenium (Se) (Martinez-Ballesta *et al.*, 2010). Based on the amount present in human body, major minerals are those present in amount more than 5 g, however, trace minerals are present in quantity less than 5 g (Kulp, 2000). Minerals are required in the body for activation of enzymes performing various metabolic functions and must be supplied from the diet as the body can't synthesize them (Škrbić *et al.*, 2009; Hussain *et al.*, 2010). Calcium is an essential constituent of teeth and bones. Additionally, it plays an important role in enzyme activation, blood coagulation, and membrane permeability. Potassium is involved in glycogenesis, regulation of osmotic pressure, and acid-base balance. It also acts as a primary cation in intracellular fluid and performs many cell membrane functions. Sodium has almost similar functionalities to that of potassium, however, it acts as a principal cation in



extracellular fluids. Magnesium is essentially required for several enzyme systems in the citric acid cycle and oxidative phosphorylation making it an important component required for the human body. The role of phosphorus in formation and breaking of high energy bonds (ATP) and synthesis of phospholipids/phosphoproteins make it an important constituent in the human body. Besides this, it acts as an essential element in bones and teeth. Cobalt is a constituent of vitamin B₁₂ and is required as a cofactor of enzymes for biosynthesis of DNA and amino acid metabolism. Selenium is known to have antioxidative properties because of its synergistic effects with alpha tocopherol's (vitamin E) antioxidative properties and is involved in the prevention of liver necrosis, white muscle disease, scavenging free radicals and oxidative damage to the cell membrane (Soetan *et al.*, 2010). Iron contributes to wide-ranging metabolic processes (e.g., oxygen and electron transport and DNA synthesis) and is considered as a biologically vital element in the daily diet of humans (Abbaspour *et al.*, 2014). Globally iron deficiency ranks 1st and comprehends a wide range of diseases including anemia and other neurodegenerative diseases (Gupta, 2014). The structural and catalytic role of zinc in wide-ranging variety protein makes it an important micronutrient in the daily diet of human beings. Globally zinc deficiency ranks 3rd and disorders are related to nervous system syndromes, reduced immunity, and impaired growth (Maret, 2012). Recently, micronutrient deficiency (e.g., Fe, Zn) was highlighted as global public health problem affecting more than 2 billion people (Guzmán *et al.*, 2014) and in this situation not meeting minimum daily requirements of micronutrients might severely affect public health (Myers *et al.*, 2014). Micronutrient deficiency is one of the most important challenges in developing countries as 32% of natives suffer from one or other of the numerous types of malnutrition (WHO, 2000). Different strategies like supplementation, fortification and diet diversification are in practice to combat micronutrient deficiencies (Darnton-Hill and Nalubola, 2002). Diet diversification by introducing local or regional foods can not only provide prospective solution to cope with malnutrition but will also strongly influence the regional sustainable development (Ramakrishnan, 2002). In developing countries like Pakistan, cereals are consumed as staple foods and can be explored as vital source of minerals in daily diet. Cereals like whole-grain barley flour based food products can contribute as a vital source of these micronutrients in daily diet. Barley varieties greatly differs in their chemical composition because of genetic variations along with environmental factors or their combine effects (Andersson *et al.*, 1999; Rodehutschord *et al.*, 2016). So, we need to figure out those barley varieties having high nutritional profile. Following literature shows that a very limited studies have been conducted previously to analyze the varietal differences in the mineral profile of barley varieties

cultivated in other countries but so far no such research has been conducted in Pakistan.

Chemical composition and physical attributes of cereal grains are strongly affected by environmental factors such as temperature, rainfall, soil conditions, and fertilization (Rodehutschord *et al.*, 2016). Keeping in view the impact of genotype and climatic conditions on the mineral concentration of cereal crops; Vignola *et al.* (2016) stated a more pronounced and strong effect of climatic conditions in comparison to genotypes. Soil mineral composition as well as climatic conditions during the deposition of mineral in each grain fraction effect variability of minerals in different varieties. Chappell *et al.* (2017) analyzed the effect of year, variety, and growing conditions on the mineral concentration of barley varieties. Considerable variations in mineral content of barley varieties were detected due to variety and year, however, no effect was observed for location. This study specified the significance of choosing varieties for particular end-use due to a significant variety of differences in most of the barley minerals. Weather conditions and growing year also influence the mineral content in cereal crops. Lahouar *et al.* (2017) analyzed the mineral profile of different barley varieties grown in Tunisia. The study indicated that barley variety "Rihane" having the highest level of analyzed minerals can provide an appreciable quantity of minerals required for adequate nutrition. Kenzhebayeva *et al.* (2019) suggested that characterizing different varieties/lines for their nutritional attributes including minerals could be useful to develop mutant varieties with appropriate levels of minerals. This can then lead to variety based product development having an appropriate level of desired minerals to overcome mineral deficiencies.

Barley is an underutilized and neglected food crop in Pakistan with a production yield of 58 thousand tones during 2016-17 (GOP, 2018). Although the importance of these minor constituents as minerals is of significant importance for human nutrition, the micronutrients of different Pakistani barley varieties have not been studied up till now. Therefore, characterizing different barley varieties for their mineral composition will help to select the best varieties with high mineral profiles to be used in barley-based food products. This study aimed to characterize the mineral profile of Pakistani grown barley varieties from different geographical regions. The study will provide the information to food processors to promote the utilization of barley varieties with high mineral profile to be incorporated in barley based food products.

MATERIALS AND METHODS

Procurement of raw material: Barley grains of twenty varieties/lines were procured from different ecological regions during 2017-2018. The samples were stored in labeled plastic jars at room temperature to ensure preserve integrity.

All the available barley varieties/lines in four provinces of Pakistan were procured (Fig. 1). Haider-93, Jau-17, and Sultan-17 were procured from Ayub Agriculture Research Institute (AARI), Faisalabad, Punjab; Rakhshan-10 and Sanober-96 were procured from Balochistan Agricultural Research and Development Center (BARDC), Quetta, Balochistan; AAJ-2013, Bajwar-2000, Jau-e-Paghmbari, MPT-V2, MPT-V4, MPT-V5, MPT-V6, MPT-V7, MPT-V9, MPT-V11, MPT-V12 were procured from Cereal Crops Research Institute, Pirsabak, Nowshera, Khyber Pakhtunkhwa; Sadabahar, Upcoming-1, Upcoming-2 and Upcoming-3 were procured from Barley and Wheat Research Institute, Hyderabad, Tandojam, Sindh.

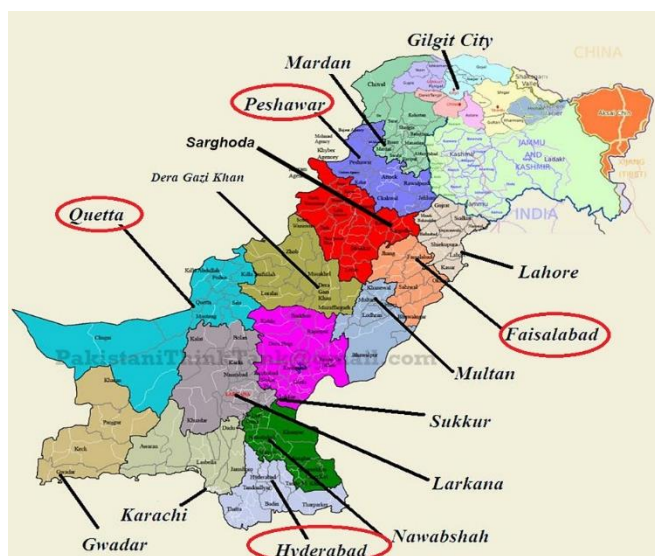


Figure 1. Map of Pakistan showing locations (red encircled) where barley varieties collected (Source: Pakistan Think Tank)

Reagents: The analytical grade reagents and chemicals were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan). The distilled water used in experiment was demineralized by passing through Millipore Milli-Q water purification system (Waters Corporation, Milford, MA, USA). Working standards were prepared by using a commercially available multi-mineral standard solution (100 mg/L, Spex CertiPrep, USA) for calibration purpose.

Sample preparation: The grains were dehulled using a laboratory-scale barley dehuller and milled to flour by a laboratory-scale milling machine (Perten Instruments Mill 3100).

Ash content: Ash percentage in different barley varieties was measured following AACC (2000) method no. 08-01. For this purpose, a flour sample (5 g) was taken in pre-weighed crucibles and charring was done directly on the flame (Bunsen burner) till fumeless. Ignition of charred samples was done in a muffle furnace (550-600°C for 5-6 hours) until the

appearance of greyish white residues and ash percentage in each variety was computed using the following formula:

$$\text{Ash(\%)} = \frac{\text{Weight of ignited residues}}{\text{sample weight}} * 100$$

Mineral profile: Ash solution was prepared following Araujo *et al.* (2008) with some modifications. Four-gram sample was placed in a porcelain crucible and heated at 600°C in muffle furnace overnight. The resulting ash was dissolved in 0.5 mL Nitric Acid Trace Metal Gold (Merck Germany). The digested sample was then transferred to 10 mL volumetric flask and volume was made up to mark by adding demineralized distilled water. Blank was prepared using similar experimental procedures and conditions eliminating analyte sample. Samples and standard solutions were analyzed for minerals using inductively coupled plasma-optical emission spectrometer (ICP-OES) (Perkin Elmer, optima 4300 Dv, USA) with Argon plasma, at the Department of Nutrition Science, Purdue University, USA, under conditions shown in Table 1. ICP-OES parameters like argon gas flow, plasma stabilization, and most appropriate wavelength for each mineral were selected. Electronic calibration of ICP OES was done routinely for validity of test results. NIST traceable mineral standards were used where Certificate of Analysis was provided by Spex CertiPred for standards and internal standards were approved by Quality Control. Calibration curves were generated using standard of each mineral element analyzed. Continuing Calibration Blank (prepared at the midpoint of each calibration) were run for quality control to monitor the long term stability of the instrument. Mineral contents in different barley varieties were acquired directly from calibration curves after correcting absorbance using an appropriate reagent blank (Skujins, 1998).

Table 1. Working conditions for ICP-OES

Instrument	ICP-OES (Perkin Elmer, optima 4300 Dv, USA)
Power	40 MHZ (Free running solid-state RF generator)
Plasma gas flow	15 L/min
Auxillary gas flow	0.8 L/min
Reading time	3 sec

Statistical analysis: All analyses were carried out in triplicate and mean values are presented as mean ± standard deviation (SD). The obtained results were statistically analyzed using a completely randomized design (CRD) using Statistix 8.1 software. To check the level of significance, analysis of variance technique (ANOVA) was applied, followed by Tukey’s Honestly Significant Difference (HSD) multiple comparison test for separation of mean values at $p \leq 0.05$. Hierarchical cluster analysis (HCA) was performed using SPSS software (IBM SPSS Statistics 22) while Principal

component analysis (PCA) was performed using The Unscrambler X 10.5.1 software.

RESULTS

Ash content: Ash percentage of flour is associated with mineral content present in flour. It is an important indicator of flour quality and is directly linked with the bran percentage of flour. Soil composition and characteristics, genetic makeup, climatic conditions, and milling process are predominant factors influencing the ash content of cereal flours (Kassegn, 2018). Statistical analysis on different barley varieties showed highly significant differences for ash percentage among different varieties. Mean values for ash content of different barley varieties have been presented in Fig. 2. Ash content in different barley varieties ranged between 1.32±0.04-2.00±0.05% with Rakhshan-10 possessing highest ash content and MPT-V6 having the lowest. Ash content in KPK varieties ranged from 1.32±0.04% to 1.85±0.05%. Among Punjab varieties, the values of ash content ranged from 1.59±0.04% to 1.80±0.05%. Barley varieties procured from Balochistan province comprised ash content ranging from 1.71±0.05% to 2.00±0.05%. The ash content in Sindh varieties ranged from 1.51±0.04% to 1.61±0.04%.

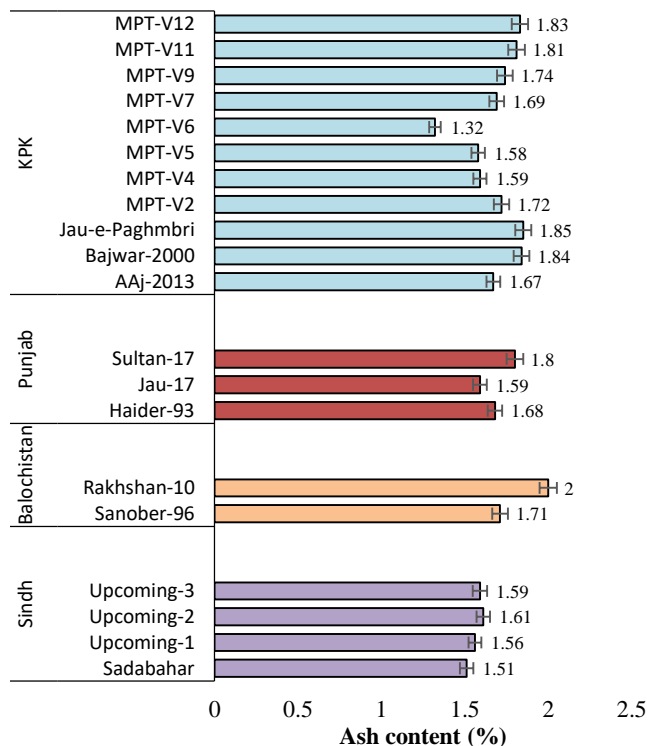


Figure 2. Ash content of different barley varieties. Error bars represent standard error (SE) of the mean; Different color bars presenting mineral content of different provinces {Blue: KPK (Khyber Pakhtoonkhwa); Red: Punjab; Green: Balochistan; Yellow: Sindh}

Mineral profile: The mineral profile of different Pakistani grown barley varieties has been presented in Table 2. Calcium concentration in different Pakistani grown barley varieties ranged from 34.0±0.3 mg/100g to 56.3±0.8 mg/100g with maximum content observed in MPT-V11 and minimum in MPT-V6. Among KPK varieties calcium content ranged from 34.0±0.3 mg/100g (MPT-V6) to 56.3±0.8 mg/100g (MPT-V11). Calcium content in Punjab varieties ranged from 36.1±0.5 mg/100g (Jau-17) to 41.3±0.6 (Sultan-17). The values for calcium content ranged from 39.6±0.6 mg/100g (Sanober-96) to 42.6±0.6 mg/100g (Rakhshan-10) in Balochistan varieties. Among Sindh varieties, calcium content ranged from 34.3±0.2 mg/100g (Upcoming-1) to 36.9±0.5 mg/100g (Sadabahar). Province-wise variation indicated that most of the varieties grown in KPK comprised the highest calcium content. Overall the varieties grown in Sindh province were found to be low in calcium.

Potassium is one of the highest mineral found in barley. Mean values for potassium content have been presented in Table 2 demonstrating the highest quantity in Rakhshan-10 (585±1.0 mg/100g) and lowest in Sadabahar (409±0.8 mg/100g). Potassium content in KPK varieties ranged from 426±0.8 mg/100g to 549±0.4 mg/100g. Among Punjab varieties, potassium content varied between 471±0.7 mg/100g to 490±1 mg/100g. The values for potassium content in Balochistan varieties ranged from 487±0.4 mg/100g to 585±1 mg/100g. Sindh varieties comprised potassium content in the range of 409±0.8-426±0.8 mg/100g. Overall, province-wise variation indicated that the lowest potassium content was found in varieties grown in Sindh Province.

The mean values for the sodium content of different varieties (Table 2) indicated Upcoming-1 with maximum Na content (29.6±0.6 mg/100g) and MPT-V6 with the minimum (9.2±0.1 mg/100g). Among KPK varieties sodium content ranged from 12.1±0.2 mg/100g to 21.6±0.5 mg/100g. Sodium content in Punjab varieties ranged from 17.0±0.3 mg/100g to 22.6±0.4 mg/100g. The values for sodium content ranged from 22.5±0.1 mg/100g to 28.4±0.6 mg/100g in Balochistan varieties. Among Sindh varieties, sodium content ranged from 20.7±0.2 mg/100g to 29.6±0.6 mg/100g. Overall the varieties grown in Sindh province were found to be the richest source of sodium. However, the varieties grown in KPK exhibited low sodium content.

Among Pakistani grown barley varieties magnesium concentration was found highest in Jau-e-Paghmbri (12.5±0.6 mg/100g) and lowest in MPT-V6 (90.3±0.2 mg/100g) (Table 2). The magnesium content in KPK varieties ranged from 90.3±0.2 µg/g to 12.5±0.6 mg/100g. Among Punjab varieties, magnesium content varied between 99.6±0.5 mg/100g to 123±0.6 mg/100g. The values for magnesium content in Balochistan varieties ranged from 114±0.4 mg/100g to 120±0.7 mg/100g. Sindh varieties comprised magnesium content in a range of 98.2±0.4-108±0.5 mg/100g. Overall barley varieties grown in Sindh province were found

Table 2. Mineral profile of Pakistani grown barley varieties

Variety/line	Ca (mg/100g)	K (mg/100g)	Na (mg/100g)	Mg (mg/100g)	P (mg/100g)	Fe (mg/100g)	Co (µg/kg)	Se (mg/kg)	Zn (mg/100g)
AAj-2013	50.4±0.8 ^c	465±0.8 ⁱ	16.3±0.2 ^{ij}	117±0.4 ^{cd}	389±0.6 ^h	4.75±0.2 ^{cde}	16.1±1 ^{bc}	0.26±0.01 ^{abc}	2.24±0.2 ^{cdef}
Bajwar-2000	52.9±0.5 ^b	549±0.4 ^b	12.1±0.2 ^m	124±0.4 ^a	430±0.4 ^c	2.86±0.1 ^{hi}	12.6±1 ^{cd}	0.29±0.02 ^{ab}	2.22±0.2 ^{cdef}
Jau-e-Paghambri	54.3±0.8 ^b	512±1 ^d	19.9±0.4 ^h	125±0.6 ^a	429±0.9 ^d	7.87±0.3 ^b	18.1±1 ^b	0.27±0.01 ^{ab}	2.42±0.2 ^{bcd}
MPT-V2	49.2±0.5 ^{cd}	490±0.8 ^{de}	14.9±0.2 ^k	115±0.5 ^e	392±0.7 ^g	4.76±0.2 ^{cde}	9.5±0 ^{de}	0.21±0.02 ^{de}	2.47±0.2 ^{bcd}
MPT-V4	48.2±0.4 ^{de}	441±0.9 ⁱ	15.7±0.1 ^{jk}	110±0.4 ^f	349±0.5 ⁱ	4.74±0.3 ^{cde}	6.5±1 ^{efg}	0.22±0.01 ^{cde}	1.98±0.2 ^{fg}
MPT-V5	46.6±0.8 ^e	459±0.6 ⁱ	21.6±0.5 ^{ef}	117±0.8 ^c	373±0.4 ⁱ	4.86±0.3 ^{cd}	4.4±1 ^{fg}	0.22±0.02 ^{cde}	2.11±0.10 ^{d-g}
MPT-V6	34.0±0.3 ^m	426±0.8 ^m	9.2±0.1 ⁿ	90±0.2 ^j	309±0.5 ^p	4.86±0.1 ^{cd}	3.1±0 ^g	0.19±0.02 ^{ef}	2.06±0.1 ^{efg}
MPT-V7	53.1±0.8 ^b	463±0.8 ⁱ	13.8±0.3 ^l	119±0.3 ^b	364±0.7 ^k	8.34±0.4 ^b	15.4±0 ^{bc}	0.15±0.02 ^{fg}	2.32±0.1 ^{cdef}
MPT-V9	43.5±0.9 ^f	477±0.6 ^g	16.8±0.3 ⁱ	109±0.5 ^{fg}	390±0.4 ^h	4.36±0.2 ^{def}	15.4±1 ^{bc}	0.25±0.04 ^{bcd}	2.41±0.0 ^{b-e}
MPT-V11	56.3±0.8 ^a	530±0.8 ^c	13.2±0.2 ^l	122±0.6 ^a	423±0.4 ^d	4.67±0.2 ^{c-f}	8.1±0 ^{def}	0.29±0.02 ^{ab}	2.43±0.1 ^{b-e}
MPT-V12	39.5±0.6 ⁱ	457±0.6 ^k	20.2±0.4 ^h	110±0.5 ^f	369±0.4 ⁱ	9.87±0.5 ^a	24.2±1 ^a	0.04±0.01 ^j	2.07±0.0 ^{d-g}
Haider-93	38.2±0.4 ^{ij}	485±0.4 ^f	17.0±0.3 ⁱ	115±0.6 ^{de}	414±0.6 ^e	3.42±0.2 ^{gh}	4.2±0 ^{fg}	0.20±0.01 ^{de}	2.77±0.1 ^b
Jau-17	36.1±0.5 ^k	471±0.7 ^h	21.6±0.1 ^{fg}	100±0.5 ⁱ	400±0.4 ^f	3.01±0.1 ^{hi}	10.1±1 ^{de}	0.19±0.01 ^{ef}	2.53±0.1 ^{bc}
Sultan-17	41.3±0.6 ^{gh}	490±1 ^d	22.6±0.4 ^{de}	123±0.6 ^a	435±0.4 ^b	4.02±0.2 ^{efg}	ND	0.30±0.02 ^a	2.07±0.1 ^{d-g}
Sanober-96	39.6±0.6 ^{hi}	487±0.4 ^e	22.5±0.1 ^{def}	114±0.4 ^e	374±0.4 ⁱ	2.41±0.1 ⁱ	20.1±1 ^{ab}	0.06±0.01 ^{ij}	1.72±0.0 ^g
Rakhshan-10	42.6±0.6 ^{fg}	585±1 ^a	28.4±0.6 ^b	120±0.7 ^b	446±0.4 ^a	4.78±0.2 ^{cde}	12.5±3 ^{cd}	0.17±0.01 ^{efg}	3.19±0.2 ^a
Sadabahar	36.9±0.5 ^{jk}	409±0.8 ^o	27.1±0.1 ^c	102±0.4 ^h	307±0.3 ^q	4.61±0.2 ^{c-f}	ND	0.18±0.01 ^{ef}	2.07±0.0 ^{d-g}
Upcoming-1	34.3±0.2 ^{lm}	420±0.6 ⁿ	29.6±0.6 ^a	102±0.4 ^h	312±0.4 ^o	5.34±0.3 ^c	ND	0.12±0.01 ^{gh}	2.18±0.1 ^{c-f}
Upcoming-2	35.1±0.2 ^{klm}	411±0.9 ^o	20.7±0.2 ^{gh}	108±0.5 ^g	326±0.3 ^m	3.98±0.2 ^{fg}	ND	0.09±0.01 ^{hi}	2.19±0 ^{cdef}
Upcoming-3	36.0±0.3 ^{kl}	426±0.8 ^m	23.1±0.3 ^d	98.2±0.4 ⁱ	320±0.4 ⁿ	4.34±0.2 ^{def}	ND	0.22±0.01 ^{cde}	2.28±0 ^{cdef}

Means in the column having different superscript are significantly different; ND: Not Detected

to be low in magnesium. Sultan-17, Rakhshan-10, and Upcoming-2 comprised the highest magnesium concentration among barley varieties grown in Punjab, Balochistan, and Sindh province, respectively.

Along with potassium, phosphorus was found in high concentrations in barley grains. Mean values for phosphorus content of different varieties have been displayed in Table 2. Phosphorus content in Pakistani barley varieties ranged from 307±0.3 mg/100g to 446±0.4 mg/100g. Rakhshan-10 was found to be the richest source of phosphorus (446±0.4 mg/100g) among barley varieties grown in Pakistan. The lowest phosphorus content was observed in Sadabahar (307±0.3 mg/100g) and overall, the varieties grown in Sindh province were found to be low in phosphorus. Among KPK varieties phosphorus content ranged from 309±0.5 mg/100g to 430±0.4 mg/100g. Phosphorus content in Punjab varieties ranged from 400±0.4 mg/100g to 435±0.4 mg/100g. The values for phosphorus content ranged from 374±0.4 mg/100g to 446±0.4 mg/100g in Balochistan varieties. Among Sindh varieties, phosphorus content ranged from 307±0.3 mg/100g to 326±0.3 mg/100 g.

Statistical analysis regarding the iron content of different barley varieties showed highly significant differences among varieties (Table 2). Results indicated that iron content in Pakistani grown barley varieties ranged from 2.41±0.1 mg/100g to 9.87±0.5 mg/100g. Iron content in KPK varieties ranged from 2.86±0.1 mg/100g to 9.87±0.5 mg/100g. Among Punjab varieties, iron content varied between 3.01±0.1 mg/100g to 4.02±0.2 mg/100g. The values for iron content in

Balochistan varieties ranged from 2.41±0.1 mg/100g to 4.78±0.2 mg/100g. Sindh varieties comprised iron content in the range of 3.98±0.2-5.34±0.3 mg/100g. Overall province wise variation indicated that all the varieties/lines grown in KPK were found to be rich sources of iron (Fe) excluding Bajwar-2000 which showed exceptionally low iron content. All the varieties grown in Punjab province exhibited the lowest iron content. The varieties grown in KPK province can be used as a potentially good source of iron in barley-based food products.

Mean values for cobalt content of different varieties (Table 2) presented MPT-V12 with highest cobalt content (24.2±1 µg/kg) and MPT-V6 (3.13±0 µg/kg) with minimum. Cobalt was not detected in all barley varieties grown in Sindh province as well as in Sultan-17 grown in Punjab province. Among KPK varieties cobalt content ranged from 3.13±0 µg/kg to 24.2±1 µg/kg. Cobalt content in Punjab varieties ranged between 4.2±0 µg/kg to 10.1±1 µg/kg. Balochistan varieties comprised cobalt content ranging between 12.5±3-20.1±1 µg/kg.

Selenium was found in trace amounts in all barley varieties. Statistical analysis regarding selenium concentration of different barley varieties showed highly significant differences among varieties. Mean values for selenium content of Pakistani grown barley varieties ranged from 0.04±0.01 mg/kg to 0.30±0.02 mg/kg with MPT-V12 showing minimum and Sultan-17 comprising maximum selenium content (Table 2). Among KPK varieties selenium content ranged from 0.04±0.01 mg/kg to 0.29±0.02 mg/kg.

Selenium content in Punjab varieties ranged from 0.19±0.01 mg/kg to 0.30±0.02 mg/kg. The values for selenium content ranged from 0.06±0.01 mg/kg to 0.17±0.01 mg/kg in Balochistan varieties. Among Sindh varieties, selenium content ranged from 0.09±0.01 mg/kg to 0.22±0.01 mg/kg. Mean values for zinc content of different barley varieties (Table 2) indicated that zinc content in different varieties ranged from 1.72±0 mg/100g to 3.19±0.2 mg/100g with Rakhshan-10 showing maximum while Sanober-96 comprising minimum zinc content, although both varieties were procured from same province (Balochistan) and grown under same environmental conditions, however, such huge difference can be attributed to their genetic make-up. Among KPK varieties zinc content ranged from 1.98±0.2 mg/100g to 2.47±0.2 mg/100g. Zinc content in Punjab varieties ranged from 2.07±0.1 mg/100g to 2.77±0.1 mg/100g. The values for zinc content ranged from 1.72±0 mg/100g to 3.19±0.2 mg/100g in Balochistan varieties. Among Sindh varieties, zinc content ranged from 2.07±0 mg/100g to 2.28±0 mg/100g.

Chemometric analysis based on mineral profile: The clustering pattern of barley varieties based upon their mineral profile has been shown in Fig. 3. Twenty barley varieties were classified into three clusters using hierarchical cluster analysis. The first cluster grouped 14 barley varieties, the second group contained five varieties, while the third group contained only one barley variety as shown in Table 3.

Table 3. Classification of barley varieties based on their mineral profile

Clusters	Barley varieties
1	AAJ-2013, Bajwar-2000, Jau-e-Paghmbri, MPT-V2, MPT-V4, MPT-V5, MPT-V7, MPT-V9, MPT-V11, MPT-V12, Jau-17, Sultan-17, Haider-93, Sanober-96
2	MPT-V6, Sadabahar, Upcoming-1, Upcoming-2, Upcoming-3
3	Rakhshan-10

These clusters can be identified in the dendrogram obtained by the centroid clustering method (Fig. 3). The separation of Sindh varieties (Sadabahar, Upcoming-1, Upcoming-2, and Upcoming-3) into separate main clusters indicates the least variation among these varieties as these varieties exhibited low mineral profile in comparison to varieties procured from other provinces. The separation of barley variety Rakhshan-10 into separate main clusters indicates its distinct nature from the rest of varieties as the mineral profile of Rakhshan-10 was found to be very high and most of the minerals in this variety were found to be significantly higher than others.

The classification ability of principal component analysis (PCA) has been presented in Fig. 4a. Overall, the first two principal components explained 97% variability; PC-1 explained 93% while PC-2 described 4%. Assessment of

score plot (Fig. 4a) shows that barley varieties Sadabahar, Upcoming-1, Upcoming-2, and Upcoming-3 were grouped along PC-2 direction and differed from the rest of varieties based upon their mineral profile. MPT-V6 (KPK variety) was also found to be closely related to Sindh varieties based on its mineral profile. Barley variety Rakhshan-10 separated along the positive direction of PC-1 and showed a high degree of dissimilarity from Sadabahar, Upcoming-1, Upcoming-2, Upcoming-3, and MPT-V6. The loading plot has been shown in Fig. 4b indicating the influence of different minerals on the PCA model as well as providing information regarding the correlation between different studied minerals. The minerals residing close to each other are positively correlated and those laying apart are negatively correlated. All the minerals separated along the positive side of PC-1 except iron and sodium. Phosphorus and magnesium were found to have a more positive correlation. Also, both these minerals were positively correlated with potassium. Iron and sodium inclined to reside in the center of loading plot indicating their least impact to cause variation in studied barley varieties based upon their mineral profile.

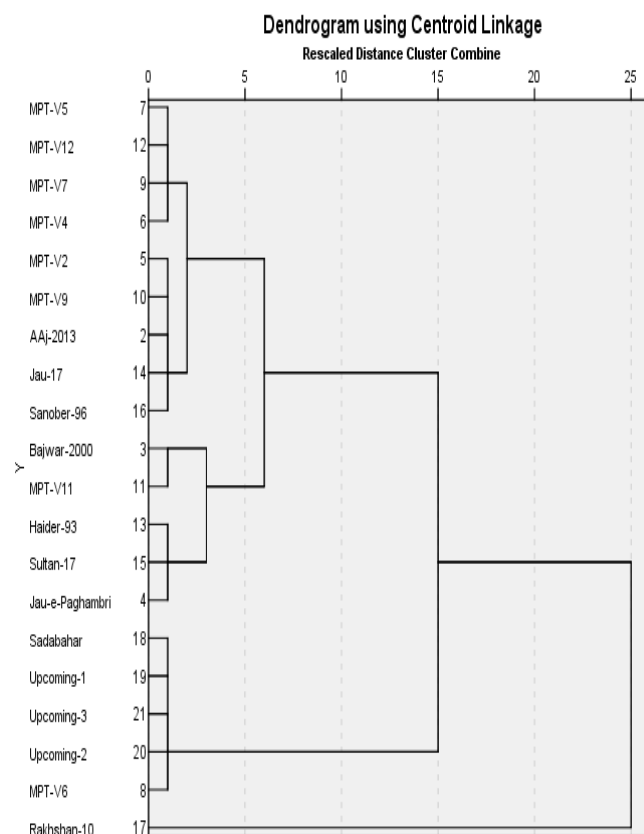


Figure 3. Clustering pattern of 20 barley varieties based upon their mineral profile; varieties lying close to each other are more closely related; clustering pattern or classification of barley varieties in different clusters is presented in Table 3.

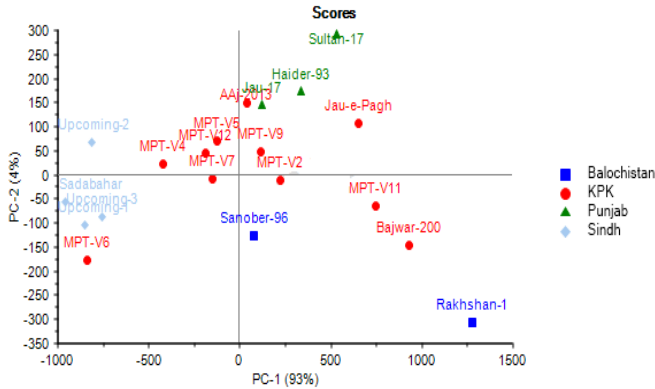


Figure 4a. Score plot of mineral profile of 20 barley varieties; figure legend symbols denoting different provinces of Pakistan and the respective symbol in the score plot presenting barley varieties of the respective province

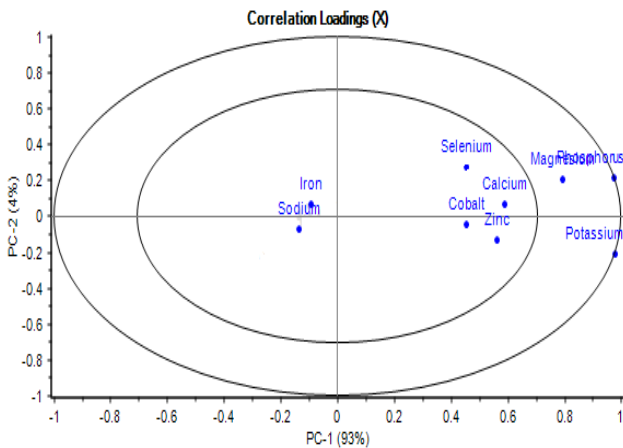


Figure 4b. Loading plot of mineral profile of 20 barley varieties; indicating influence of different minerals (shown with blue dots) on Principal Component Analysis (PCA) model

DISCUSSION

Current research was planned to characterize the mineral profile of Pakistani grown barley varieties from different geographical regions. Ash percentage of flour is associated with mineral content present in flour. Results regarding ash content of Pakistani barley varieties are supported by some previous research discussed hereafter: Erkan *et al.* (2006) found 1.31% ash content in hullless barley flour. Abeshu and Abrha (2017) reported ash content ranging 1.43% to 2.34% in different barley varieties grown in Ethiopia. Chappell *et al.* (2017) reported 1.0-2.2 g per 100 g ash content in different barley varieties and indicated that variations in ash content of different barley varieties are allied to the difference in growing locations, agronomic factors as well as growing year. The ash content (1.0-1.2%) in different barley varieties (waxy

and regular starch type) was found to be statistically non-significant as reported in a study by Vasanthan *et al.* (2002). Wu *et al.* (2000) observed 1.48% ash in hullless barley. Results regarding ash content of Pakistani barley varieties is in line with the findings of above mentioned literature. Dyulgerova *et al.* (2017) reported 2.5-2.89% ash content in 21 six-row winter barley varieties cultivated in Bulgaria which is slightly higher than the ash content of Pakistani barley varieties.

Literature shows that a very limited studies (discussed hereafter) have been conducted previously to analyze the varietal differences in the mineral profile of barley varieties cultivated in other countries. Chappell *et al.* (2017) analyzed mineral concentration of different barley varieties grown in Orkney and showed following concentrations: 28.2 to 43.2 mg/100g calcium, 474.6 to 559.4 mg/100 g potassium, 7.6-17.4 mg/100 g sodium, 104.0 to 131.2 mg/100 g magnesium, 352.2 to 472.1 mg/100 g phosphorus, 3.59 to 6.48 mg/100 g iron, 2.10 to 3.72 mg/100 g zinc. Lahouar *et al.* (2017) reported following mineral profile of barley varieties grown in Tunisia: 62.80±0.05 to 70.29±0.07 mg/100 g calcium, 313.00±0.55 to 480.00±3.6 mg/100 g potassium, 78.00±0.11 to 194.00±0.20 mg/100 g sodium, 49.572±0.86 to 71.19±0.37 mg/100 g magnesium, 440.00±6.08 to 570.00±2.88 mg/100 g phosphorus, 0.098 mg/100 g iron. Youssef *et al.* (2013) stated following concentration of mineral in barley grains of Egypt: 120-140 mg/100 g calcium, 290-320 mg/100 g potassium, 43.25-47.35 mg/100 g sodium, 150-180 mg/100 g magnesium, 300-370 mg/100 g phosphorus, 8.41-8.96 mg/100 g iron, 5.67 mg/100 g zinc. Demirbas (2005) reported 0.14±0.05% calcium, 2.51±0.3% potassium, 0.24±0.06% magnesium, 0.27±0.08% phosphorus, 28.7 mg/kg iron, 42.4±3.8 mg/kg zinc in barley grains grown in Turkey.

Following section discusses the findings of present study in comparison to literature cited in above paragraph. The calcium content in different barley varieties as reported by Chappell *et al.* (2017) is in line with the calcium content of Pakistani barley varieties. However, other studies in above cited literature (Youssef *et al.*, 2013; Lahouar *et al.* 2017) reported much higher calcium content in comparison to calcium content reported in present study. Results regarding sodium and potassium content in Pakistani grown barley varieties are in agreement with Chappell *et al.* (2017), however, Youssef *et al.* (2013) and Lahouar *et al.* (2017) reported much higher sodium content and lower potassium content in comparison to present study. The higher concentration of sodium is attributed to relatively low potassium content reported in these studies as concentration of both these minerals is often reciprocal to each other (Mahmood *et al.*, 2012). Magnesium content reported by Youssef *et al.* (2013) and Chappell *et al.* (2017) in the literature cited above is slightly higher than findings of the present study, however, magnesium content of Pakistani barley varieties is in good agreement with Lahouar *et al.*

(2017). The phosphorus content of Pakistani grown barley varieties is in accordance with phosphorus content reported in above cited literature (Demirbas, 2005; Youssef *et al.*, 2013; Chappell *et al.*, 2017; Lahouar *et al.*, 2017). Some barley varieties in the present study specially those grown in Khyber Pakhtoonkhwa province shown appreciable quantity of iron content. Results regarding iron and zinc content of Pakistani barley varieties are in line with Demirbas (2005), Youssef *et al.* (2013) and Chappell *et al.* (2017). However, Lahouar *et al.* (2017) found 0.098 mg/100 g in Rihane barley (a variety grown in Tunisia) which is much lower than iron content found in the present study. Results of the present study indicated that all analyzed minerals varied significantly among different varieties. Although, both environmental conditions and genetic make-up influenced the mineral profile of different varieties, however, environmental conditions especially soil composition had a more pronounced effect on mineral profile (Vignola *et al.*, 2016). Different varieties have a difference in the path of uptake and mechanism for accumulation of minerals influencing variation in mineral content of varieties grown at the same environmental locations. Some varieties analyzed in the present study showed better capability in accumulating minerals in comparison to others.

According to dietary reference intake (DRI) set by Food and Nutrition Board of the National Research Council (NRC (National Research Council), 2001)) mineral requirement (mg per day) varies as follows: 3500 for potassium, 700 for phosphorus, 320-420 for magnesium, 1000 for calcium, 8-18 for iron, 0.9 for copper, 8-11 for zinc, 0.055 for selenium. Keeping in view the mineral content of Pakistani grown barley varieties, barley flour can meet DRI requirement of potassium and phosphorus. Barley can also serve as an important natural source of selenium in daily diet. Iron and zinc DRI requirement can be met up to 50% depending upon the variety of barley as well as percentage of barley flour used in food product.

Conclusion: The present study has shown considerable variations in minerals of Pakistani grown barley varieties. Barley varieties were found to be high in potassium and phosphorus. Overall high mineral profile was observed for variety Rakhshan-10 grown in Balochistan province. The varieties grown in KPK province comprised high iron content. Varieties grown in Sindh province presented poor mineral profile. Incorporation of barley varieties with an overall high mineral profile (e.g., Rakhshan-10) or those with a rich source of a particular mineral (e.g., KPK varieties rich in iron) in food products possibly will contribute a greater proportion of recommended dietary allowance (RDA) of these essential micronutrients and will help to cope up with challenges of a specific micronutrient deficiency (e.g. iron-deficiency anemia). The findings of this study are also significant to provide an outlook to improve the nutritional quality of barley

and to set nutritional and export guidelines and regulations in Pakistan. Furthermore, the variation in the mineral profile of 20 Pakistani grown barley varieties can be used in the breeding programs to develop a new barley cultivar with higher mineral content hence improving the nutritional attributes of barley grains. However, to get a clear understanding of variation in chemical composition of different barley varieties either due to growing environment or cultivar one or two varieties must be grown at all locations.

Acknowledgments: The authors are thankful to the Higher Education Commission, Pakistan, to provide funding for this project through the International Research Support Initiative Program (IRSIP). The authors are also grateful to Prof. Dr. Osvaldo Campanella, Department of Agricultural and Biological Engineering and Berdine Martin, Department of Nutrition Science, Purdue University, USA for providing research facilities and support during the completion of this project.

Author contributions: Nida Kanwal conducted research, analyzed data and wrote manuscript. Imran Pasha helped in planning of experiment, supervised throughout the experiment and reviewed the manuscript. Aamir Shehzad helped in conceptualization and planning of experiment. Rizwan Shukat and Anjum Zia supervised the experiment, reviewed and edited this manuscript.

Conflict of Interest: The authors declare that there is no conflict of interest.

REFERENCES

- AACC. 2000. Approved Methods of the American Association of Cereal Chemist (AACC). 9th Ed. Washington DC, USA. Association of Official Analytical Chemist.
- Abbaspour, N., R. Hurrell and R. Kelishadi. 2014. Review on iron and its importance for human health. *Journal of Research in Medical Sciences* 19:164-174.
- Abeshu, Y. and E. Abrha. 2017. Evaluation of proximate and mineral composition profile for different food barley varieties grown in central highlands of Ethiopia. *World Journal of Food Science and Technology* 1:97-100.
- Andersson, A.A.M., C. Elfverson, R. Andersson, S. Regne and P. Aman. 1999. Chemical and physical characteristics of different barley samples. *Journal of the Science of Food and Agriculture* 79:979-986.
- Araujo, R.G.O., S.M. Macedo, M.G.A. Korn, M.F. Pimentel, R.E. Bruns and S.L.C. Ferreira. 2008. Mineral composition of wheat flour consumed in Brazilian cities. *Journal of the Brazilian Chemical Society* 19:935-942.

- Baik, B.K. and S.E. Ullrich. 2008. Barley for food: Characteristics, improvement, and renewed interest. *Journal of Cereal Science* 48:233-242.
- Chappell, A., K.P. Scott, I.A. Griffiths, A.A. Cowan, C. Hawes, J. Wishart and P. Martin. 2017. The agronomic performance and nutritional content of oat and barley varieties grown in a northern maritime environment depends on variety and growing conditions. *Journal of Cereal Science* 74:1-10.
- Darnton-Hill, I. and R. Nalubola. 2002. Fortification strategies to meet micronutrient needs: Successes and failures. *Proceedings of the Nutrition Society*. 61:231-241.
- Demirbas, A. 2005. β -glucan and mineral nutrient contents of cereals grown in Turkey. *Food Chemistry* 90: 773-777.
- Dyulgerova, B., N., Dyulgerov and D. Dimova. 2017. Variation in the chemical composition and physical characteristics of grain from winter barley varieties. *Agricultural Science and Technology* 9:198-202.
- Erkan, H., S. Celik, B. Bilgi and H. Koksel. 2006. A new approach for the utilization of barley in food products: barley tarhana. *Food Chemistry* 97:12-18.
- FAO. 2018. FAOSTAT (Food and Agriculture Organization of the United Nations). Crops [Internet]. 2018. Available from:
- GOP. 2018. Government of Pakistan Finance Division, Economic Advisor's Wing, Islamabad, Pakistan.
- Gupta, D.C. 2014. Role of Iron (Fe) in body. *IOSR Journal of Applied Chemistry* 7: 8-46.
- Guzmán, C., A.S. Medina-Larqué, G. Velu, H. González-Santoyo, R.P. Singh, J. Huerta-Espino, I. Ortiz-Monasterio and R.J. Peña. 2014. Use of wheat genetic resources to develop biofortified wheat with enhanced grain zinc and iron concentrations and desirable processing quality. *Journal of Cereal Science* 60:617-622.
- Hussain, A., H. Larsson, R. Kuktaite and E. Johansson. 2010. Mineral composition of organically grown wheat genotypes: contribution to daily minerals intake. *International Journal of Environmental Research and Public Health* 7:3442-3456.
- Kassegn, H.H. 2018. Determination of proximate composition and bioactive compounds of the Abyssinian purple wheat. *Cogent Food and Agriculture* 4:1421415.
- Kenzhebayeva, S., A. Abekova, S. Atabayeva, G. Yernazarova, N. Omirbekova, G. Zhang, S. Turasheva, S. Asrandina, F. Sarsu and Y. Wang. 2019. Mutant lines of spring wheat with increased iron, zinc, and micronutrients in grains and enhanced bioavailability for human health. *BioMed Research International* 2019:1-10.
- Kulp, K. 2000. *Handbook of Cereal Science and Technology*, revised and expanded, CRC Press. ISBN 9780824782948.
- Lahouar, L., F. Ghrairi, A. El Arem, S. Medimagh, M. El Felah, H.B. Salem and L. Achour. 2017. Biochemical composition and nutritional evaluation of barley rihane (*Hordeum vulgare* L.). *African Journal of Traditional Complementary and Alternative Medicine* 14:310-317.
- Mahmood, T., F. Anwar, T. Iqbal, I.A. Bhatti and M. Ashraf. 2012. Mineral composition of strawberry, mulberry and cherry fruits at different ripening stages as analyzed by inductively coupled plasma-optical emission spectroscopy. *Journal of Plant Nutrition* 35:111-122.
- Maret, W. 2012. New perspectives of zinc coordination environments in proteins. *Journal of Inorganic Biochemistry* 111:110-116.
- Martinez-Ballesta, M.C., R. Dominguez-Perles, D.A. Moreno, B. Muries, C. Alcaraz-Lopez, E. Bastias, C. Garcia-Viguera and M. Carvajal. 2010. Minerals in plant food: effect of agricultural practices and role in human health. A review. *Agronomy for Sustainable Development* 30:295-309.
- Myers, S.S., A. Zanobetti, I. Kloog, P. Huybers, A.D. Leakey, A.J. Bloom, E. Carlisle, L.H. Dietterich, G. Fitzgerald and T. Hasegawa. 2014. Increasing CO₂ threatens human nutrition. *Nature* 510:139-142.
- NRC. 2001. National Research Council. *Dietary Reference Intakes table – The complete set*
- Ramakrishnan, U. 2002. Prevalence of micronutrient malnutrition worldwide. *Nutrition Reviews* 60:S46-52.
- Rodehutsord, M., C. Rückert, H.P. Maurer, H. Schenkel, W. Schipprack, K.E.B. Knudsen, M. Schollenberger, M. Laux, M. Eklund, W. Siegert and R. Mosenthin. 2016. Variation in chemical composition and physical characteristics of cereal grains from different genotypes. *Archives of Animal Nutrition* 70:87-107.
- Škrbić, B., S. Milovac, D. Dodig and B. Filipčev. 2009. Effects of hull-less barley flour and flakes on bread nutritional composition and sensory properties. *Food Chemistry* 115:982-988.
- Skujins, S. 1998. *Handbook for ICP-AES (Varian-Vista). A short guide to Vista Series ICP-AES operation*. Zug, Switzerland: Varian Int. AG.
- Soetan, K.O., C.O. Olaiya and O.E. Oyewole. 2010. The importance of mineral elements for humans, domestic animals and plants-A review. *African Journal of Food Science* 4:200-222.
- Sullivan, P., E. Arendt and E. Gallagher. 2013. The increasing use of barley and barley by-products in the production of healthier baked goods. *Trends in Food Science and Technology* 29:124-134.
- Vasanthan, T., J. Gaosong, J. Yeung and J. Li. 2002. Dietary fiber profile of barley flour as affected by extrusion cooking. *Food Chemistry* 77:35-40.
- Vignola, M.B., M. Moiraghi, E. Salvucci, V. Baroni and G.T. Pérez. 2016. Whole meal and white flour from Argentine

- wheat genotypes: mineral and arabinoxylan differences. *Journal of Cereal Science* 71:217-223.
- WHO. 2000. World Health Organization. Nutrition for health and development. A global agenda for combating malnutrition. Available online at: <http://www.who.int/mip2001/files/2231/NHDprogressreport2000.pdf>
- Wu, J.F., C. Cheng, I. Yu and J. Hsyu. 2000. Hulless barley as an alternative energy source for growing–finishing pigs on growth performance, carcass quality, and nutrient digestibility. *Livestock Production Science* 65:155-160.
- Youssef, M.K.E., F.A.E. El-Fishawy, E.A.E. Ramadan and A.M.A. El-Rahman. 2013. Nutritional assessment of barley, talbina and their germinated products. *Frontiers in Science* 3:56-65.