Comparative analysis of mineral content and proximate composition from chilli pepper (*Capsicum annuum* L.) germplasm

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Citation
Nadra Khan, Muhammad Jamil Ahmed and Syed Zulfiqar Ali Shah. Comparative analysis of mineral content and proximate composition from chilli pepper (*Capsicum annuum* L.) germplasm. Pure and Applied Biology. Vol. 8, Issue 2, pp 1338-1347. http://dx.doi.org/10.19045/bspab.2019.80075

Received: 26/02/2019 Revised: 24/04/2019 Accepted: 29/04/2019 Online First: 30/04/2019

Abstract
The study was conducted with the objective to estimate the nutritional composition from 35 diverse genotypes of (*Capsicum annuum* L.) which were previously uninvestigated, for selection of highly nutritive genotypes as a parent for further hybridization program. The genotypes were divided into three groups. Group I (thin type), group II (intermediate) and group III (cherry chillies). Mineral contents calcium, iron, magnesium, manganese, zinc and copper were analysed using atomic absorption whereas potassium and sodium were estimated through flame photometer. Phosphorus was determined using a spectrophotometer. Moisture, protein, fat, ash and crude fiber contents were estimated by the AOAC method, while carbohydrate content was estimated by the difference method. In relation to macro minerals highest magnesium (287.52 mg/100), calcium (114.0 mg/100g) and potassium (286.30 mg/100g) was observed in group II, whereas phosphorus was highest in group I (29.20 mg/100g). In the case of micro minerals highest iron (109.0 mg/100g) and copper (0.21 mg/100g) content were depicted in a group III, sodium (15.90 mg/100g) in group II, while manganese (0.50 mg/100g) and zinc (0.20 mg/100g) were highest in group I. As far as concern proximate composition, fats (2.15 %), ash (8.93 %) and protein (9.09 %) was maximum in group III. Moisture (11.86) and carbohydrates (52.28 %) was highest in group II. Based on these results chilli peppers contain a high amount of magnesium, potassium, calcium and iron content, these chilli peppers could be preferably selected for the breeding purpose for the further crop improvement program.

Keywords: *Capsicum annuum* germplasm; Macro minerals; Micro minerals; Proximate composition

Introduction
*Capsicum* is a genus of plants belonging to the nightshade family *Solanaceae*, tribe Solanaceae, subtribe Capsicinae [1]. It is the oldest cultivated crops of the Americas [2]. It is the most produced type of spice used for coloring and flavoring food as well as providing minerals and vitamins [3]. Red peppers are the berries of *Capsicum* plant and these are used for culinary purposes as an essential ingredient of the culinary throughout the world [4]. The dried whole fruits or fresh green or their powder with varying degrees of pungency, flavor and aroma, exists great scope for its export. Chilli is a vital ingredient in Pakistani dishes pickles, chutnies and curries. Besides being a vital food crop, it is also used for a pharmaceutical purpose [4]. Peppers are used in the form of ground...
powder, although oleoresins are extensively used today [5]. It is very remunerative and brings worthy profits to the farmers. It is produced seasonally but consumed throughout the year. During the year 2016-17, the overall production of Capsicum in Pakistan was 143.1 thousand tons. Nearly 64.2 thousand hectares of land area is used for its cultivation [6]. Badin, Mirpur khas and Umerkot, districts of Sindh represent the national clusters of red chillies production. Kunri, a small town in Umerkot, contributes around 86% of chillies and is rightly known as one of the largest production centers for red chillies in Asia [7]. Land of Pakistan blessed with diverse type of soil and climate that grow numerous exogenous and indigenous spices, herbs, medicinal plants and oil bearing plants. Regardless of the availability of the diverse agro-climatic conditions of the country, to produce this extensive range of plant species and as they were playing an important role in the national economy, through producing substantial export earnings or import exchange, the study conducted on their mineral content and proximate composition is limited. Nutritional data on these genotypes of pepper will be valuable for the nutritional education of the people as a means to improve the dietetic status of the population. This study was therefore conducted to generate information on the nutritional composition of the Capsicum genotypes for selection of highly nutritious genotypes for further breeding programme.

Materials and methods

Sample collection and preparation

The plant material comprised of 35 pepper (Capsicum annuum) genotypes of three types shown in (Figure 1) along with their id and country of origin shown in (Table 2), were obtained from National Gene Bank, National Agricultural Research Centre (NARC), Islamabad. Samples were washed with tap water to remove all kind of dirt, rinsing was done with distilled water, fruits were sliced in almost uniform size to facilitate drying of sliced pieces were then kept in an oven at 60 °C for 30 hours, until they were hard. At this stage, no microorganisms were grown and precaution was taken to avoid any kind of contamination.

Extraction procedure for minerals

The procedure was adopted by [8] with slight modification, was used for the digestion of the pepper samples. Peppers were dried in air, for 2 days for removal of accesses of moisture then dried in an oven for 2-3 days at 60 °C. Oven-dried pepper (Capsicum annuum) samples were ground using grinder into a fine powder. A ground pepper sample of 0.25g was weighed into 100ml conical flask, weighing balance Mettler Toledo AE 163 (Zurich, Switzerland), followed by the addition of 10 mL of an acid mixture of perchloric acid and nitric acid in the ratio of 1:2. A flask was kept on a hot plate (heater) under a fume hood and heated slowly until frosting ceased and the foaming stopped. Then the heat was increased until the boiling of mixture get started. Boiling was maintained until a clear solution was obtained. The heat was removed, samples were allowed to cool down, filtered and then diluted to mark with distilled water in a 50 mL-capacity volumetric flask. The samples were then analysed for the concentrations of calcium, iron, magnesium, manganese, zinc and copper, using atomic absorption spectrophotometer (Perkin Elmer, Salem, MA, USA). Three replicate analyses were carried out for every sample under study. Potassium (K) and Sodium (Na) were analysed with corning 400 flame photometer. Phosphorus was determined spectrophotometer (JASCO V-630). The concentration of phosphorus was determined through the measurement of the yellow phosphor vanado-molybdate complex using Cecil Digital Spectrophotometer series.

Estimation of proximate composition

Completely ripe fruits were harvested from the field of National Agriculture Research Centre, Islamabad. Fruit data of genotypes
were collected and transferred to the Laboratory of Food Science and Technology, The University of Poonch Rawalakot, AJK, for chemical analysis. An edible portion (pulp) of fruit was analysed for: Moisture, protein, fat, ash and crude fiber contents were estimated by AOAC method [9] whereas carbohydrate content was estimated by difference: 100- (%Fat + %Ash+ %Moisture + %Protein + %Crude fiber). The Soxhlet extraction method was used for estimation of fat content by extracting 2g of the sample with petroleum ether (boiling point of 40 °C to 60 °C). For estimation of ash content was determined by weighing 2g of dry a sample into a porcelain crucible which was burned at 550 °C in muffle furnace until ash was attained. The moisture content was evaluated by hot air oven method at 105 °C. The Kjeldahl method was used for the estimation of protein content. The crude fiber was determined by thorough extraction of soluble substances in sample using and 1.25% NaOH and 1.25% H₂SO₄ acid solution after the residue was ashed and the loss in weight was recorded as crude fiber.

| Serial no. | Genotype ID | Country of Origin |
|------------|-------------|-------------------|
| **Group I** |             |                   |
| 1          | 36637       | China             |
| 2          | 36635       | Germany           |
| 3          | 36620       | Bulgaria          |
| 4          | 36588       | Bulgaria          |
| 5          | 36622       | Japan             |
| 6          | 36624       | Hungary           |
| 7          | 36636       | Hungary           |
| 8          | 36592       | USA               |
| 9          | 36597       | Bhutan            |
| 10         | 36591       | Bulgaria          |
| 11         | 36632       | Netherlands       |
| 12         | 36617       | India             |
| **Group II** |          |                   |
| 13         | 36616       | Netherlands       |
| 14         | 36628       | Japan             |
| 15         | 36612       | Japan             |
| 16         | 36114       | Asgrow            |
| 17         | 36649       | Democratic Republic of the Congo |
| 18         | 30881       | Mexico            |
| 19         | 36570       | Bhutan            |
| 20         | 36569       | Bangladesh        |
| 21         | 36627       | Argentina         |
| 22         | 30879       | USA               |
| 23         | 36565       | Jamaica           |
| **Group III** |         |                   |
| 24         | 36560       | Cuba              |
| 25         | 33828       | Roggli            |
| 26         | 36563       | Czechoslovakia    |
| 27         | 36644       | Denmark           |
| 28         | 36645       | Japan             |
| 29         | 36658       | India             |
| 30         | 36630       | Costa Rica        |
| 31         | 30877       | Israel            |
| 32         | 36651       | Zambia            |
| 33         | 36652       | Zambia            |
| 34         | 36558       | Japan             |
| 35         | P6          | Pakistan          |
Results and discussion

Mineral determination

Macro minerals

*Capsicum annuum* may serve as a good supplement in the body supply of magnesium, calcium, iron, sodium, phosphorous, and potassium. Calcium in conjugation with phosphorous, manganese and magnesium, ascorbic acid, vitamin A, D, protein and chlorine involved in the formation of bone [10]. Although, they contain a small portion of the whole diet, micro and macro minerals play an important part in several metabolic processes, essential for keeping good health and avoidance of diseases and their deficiency is commonly harmful to normal biochemical functioning of the human body [11].

Phosphorus is an important mineral found in the human body. The highest amount of phosphorus is present in muscles and bones of the body [12]. It also assists calcium in many functions although it has its own independent functions [19]. Highest phosphorus content was seen in group I (29.20 mg/100g) followed by group III (25.60 mg/100g), lowest (20.33 mg/100g) was depicted in group II. These results are in accordance with [13, 14].

Potassium plays an imperative role in neurotransmission, regulation of heart beat and maintain water balance in the human body [15]. It is also an important nutrient and has a significant role in the synthesis of proteins and amino acids [16]. It is reported by [17] that the high amount of potassium in the body increase utilization of iron and helpful for people using diuretics to prevent hypertension and suffering from too much excretion of potassium through the body fluid. Both sodium and potassium in the body are also helpful for the prevention of high blood pressure. Highest (286.30 mg/100g) potassium was observed in group II followed by (279.0 mg/100g) seen in group III while lowest (272.37 mg/100g) was observed in group I. These results are in accordance with [14, 18].

The reason for a high amount of potassium could be genetic. The plants are probably adapted to soils with a high concentration of these nutrients and therefore had developed a natural mechanism to uptake these elements from the soil regardless of the nutritional composition of the soil.

Vegetables are considered as a principal source of calcium and their amount varies in food [12]. It plays an important role in maintaining normal excitability of heart, muscle contraction, blood coagulation [19]. Highest (114.0 mg/100g) calcium was observed in group II followed by (107.80 mg/100g) was shown in group III. Lowest (99.42 mg/100 g) calcium was seen in group I. Our findings are in accordance with [14]. The richness of this mineral in peppers depends on a range of reasons, such as the farming methods and nature of soil [20].

Magnesium is important for the release of parathyroid hormone and for its activities in the kidney, backbone, and intestine and also involved as a catalyst for converting vitamin D to its active state [17]. Its deficiency results in uncontrollable twisting of muscles leading to tetanus and convulsion which may both lead to death [17, 21]. Only 1 percent of Mg is extracellular [12]. Highest (287.52 mg/100g) magnesium was observed in group II followed by a group I (265.43 mg/100g) whereas the lowest magnesium was seen in group III (250.89 mg/100g) as shown in (Figure 2). These results are in accordance with [14].

Micro minerals

Copper is an important trace element that cannot be prepared by the human body. It must be consumed from dietary sources. Foods contribute essentially all of the copper ingested by humans. It also involved in the development of red blood cells, the utilization and absorption of iron, the metabolism of glucose and cholesterol, release and synthesis of life-sustaining enzymes and proteins. Highest copper was found in a group III (0.21 mg/100g) followed by group II (0.12 mg/100g) and...
lowest was found in group I (0.09 mg/100g). Our results are in agreement with the findings of [13, 22].

Zinc is present in the entire tissues of the body and it is a part of more than 50 enzymes [23]. It also plays an important role in cell growth, cell division, healing of a wound and the breakdown of carbohydrates. Highest (2.00 mg/100g) zinc was found in group I followed by group III (1.76 mg/100g) while the lowest was seen in group II (1.71). Our findings are in agreement with [13, 22, 24, 25].

Iron is an abundant element on earth [26]. It is biologically necessary component of every living organism [27]. Iron reported to be very essential for the normal functioning of the nervous system [28].

Iron has been reported as a vital trace element that plays many biochemical functions in the body including oxygen-binding hemoglobin and acts as a key catalytic center in many enzymes like, the cytochrome [21]. Highest (109.0 mg/100g) iron content was found in group III followed by group I (104.06 mg/100g) while lowest was observed in group II (102.82 mg/100g) as shown in (Figure 3).

Our results are in agreement with [14].

Sodium (Na) is an essential mineral that plays a significant role in the regulation of body fluid and helps in the maintenance of electric potential in the human body [15]. It also helps in the proper functioning of the muscles and nerves [17]. Highest (15.90 mg/100g) sodium was depicted in the group II followed by group III (13.17), whereas lowest was shown in group I (10.82 ng/100g). These findings are in accordance with [14, 29]. The variations in the sodium contents may be credited to factors, for example, the preferential uptake of Na by these set of genotypes as well as their requirement in formation of fruit and overall development of plant [30].

Manganese (Mn) is a vital heavy metal that is naturally occurring in the environment. Daily consumption through dietary sources delivers the required amount necessary for several physiological processes, including energy metabolism, antioxidant defense, immune function and others [31]. Highest (0.50 mg/100g) manganese contents were found in group I followed by group II (0.47 mg/100g) while the lowest was found in group III (0.32 mg/100g). These findings are in agreement with [22].

The concentrations of these vital elements may differ from each other they are influenced by numerous agricultural practices such as type of fertilizer, soil, use of herbicides and pesticides. These important elements are present in vegetables in trace and ultra-trace amounts. Hence, an analytical method with appropriate sensitivity is required for the correct determination of these minerals in their edible portions. Among these elements in our pepper sample magnesium, potassium, calcium, iron and zinc were most abundant sodium and manganese was least abundant. Capsicum annuum can make a contribution to intake of these minerals however these are normally used in small quantities in cooking. It can also serve as sources of fulfilling part of the daily requirements for iron and zinc.

The concentrations of nutrients were critically influenced by varieties of pepper [32], plant nutrition [33], fruits ripening [34] and conditions of weather during the growing period [35]. Many studies showed that the ripening stage of the fruit sample exercises a strong effect on the mineral nutrients of the pepper [25].

**Proximate composition**

Nowadays vegetables with high quality and nutritional contents are of consumer’s attention. Pepper is renowned as one of the best vegetable for human health [36]. Some workers found a higher concentration of health-promoting constituents in organic vegetables comparatively in conventional ones [37]. Therefore, understanding of the variations of proximate composition across (Capsicum annuum) germplasm has great worth in developing better-quality of
pepper varieties through crop improvement program.

Figure 1. Representative images of three different genotypic groups of *Capsicum annuum*  
A: Group I; B: Group II; C: Group III

![Figure 1](image1.png)

![Figure 2](image2.png)

Figure 2. Macro minerals of chilli pepper (*Capsicum annuum*) germplasm

![Figure 3](image3.png)

Figure 3. Micro minerals of chilli pepper (*Capsicum annuum*) germplasm

Minimum variation regarding crude fat was observed between groups. Maximum (2.15 %) fat was seen in group III followed by group II (2.10 %) while minimum (1.51
%) was shown in group I. The detailed proximate composition as shown in (Figure 4). Our findings are in accordance with the findings of [22, 38]. The high content of crude fat in pepper may not be valued as an oilseed, but, the oil might be extracted for use as an essential oil or essence [39].

Highest ash was seen in group III (9.09 %) followed by group II (7.01%) whereas, lowest was depicted in group I (6.0 %). These values agree with the findings reported by other investigators [22, 38]. The ash content increased significantly during ripening. This might be due to the fact of migration of inorganic ions from various parts of the plant to the active growth regions during ripening. The high ash contents suggest the availability of minerals in the samples which is confirmed by the significantly high content of phosphorus (29.20 mg/100g), iron (109.0 mg/100g) and copper (0.21 mg/100g) in group III. The compound present in higher quantity in vegetable and fruit is water and it helps in regulating pH, temperature and maintains the integrity of the tissue and cell of the human body [19]. Maximum moisture content was found in group II (11.86 %) followed by group III (9.67 %) while the lowest was seen in group I (7.96 %). These results correspond with the findings of [14, 22, 29]. The high levels of moisture in group II suggested that the pepper may not be stored for a long time without spoilage since high water activity might increase microbial action that leads to food spoilage. Therefore, dehydrating pepper increases the shelf-life or storage-life of peppers.

Protein has an important role in the human diet due to it provides an amino acid that cannot be produced by the body itself [12]. Crude protein is a measure of dietary protein that depends on the assumption that the average amino acid in a protein contains (16 %) nitrogen. Highest crude protein was found in group III (8.93 %) followed by a group I (7.13 %). Lowest was seen in group II (6.07 %). These results are in accordance with [38]. The high content of crude protein in pepper might be due to the presence of active proteinous metabolites, like capsaicin. Peppers need to be combined with other food stuff having high protein content to meet the requirement of protein for an individual [38].

As one of important food constituent, the fiber is useful for human fitness. A number of investigations reported by [40] demonstrated that it may prevent many diseases such as obesity, colon cancer, diabetes and cardiovascular problems. The investigation showed that highest (27.42 %) fiber was depicted in group III followed by group I (24.24 %), while the lowest (21.59 %) was found in group II. We found fiber content similar to those reported by [29]. Carbohydrate is an important constituent of peppers and commonly measured in assessing fruit marketable quality attributes. It is recognized that carbohydrates are very important in determining nutritional, flavor and technological characters in fresh as well as in processed food stuffs. The present results revealed high carbohydrate levels. Highest carbohydrates were found in group II (52.28 %), followed by group III (48.41 %) while lowest (45.05 %) was seen in group I, as shown in (Fig. 6). Our results are agreed with [22], [29]. During ripening, changes occur in the composition of the cell wall of carbohydrates, primarily as a result of the action of wall-degrading enzymes [41]. However, carbohydrates present, even high in value might not be nutritionally assessable meanwhile most of them are bound to remain undigested in the body [24]. The concentration and the composition of these elements may also differ depending on growing and weather conditions such as the method of fertilization, humus and soil complex, nutrient accessibility, etc.
Figure 4. Proximate composition of chilli pepper (Capsicum annuum) germplasm

Conclusion
The present investigation has delivered some comparative chemical information on macro, micro elements and proximate composition of pepper genotypes. There are indications that our genotypes are a good source of nutrients and mineral elements. Hence the understanding of variations of mineral and proximate composition across (Capsicum annuum) germplasm has great significance in developing the improved quality of pepper varieties via crop improvement program and offer great potential for future exploitation.

Authors’ contributions
Interpretation of data and drafting the article: N Khan, Final approval of the version: MJ Ahmed, Revising it critically: SZA Shah.

Acknowledgment
The writer wants to thanks the department of horticulture for financing and technical support. Authors also thanks to the Laboratory of Food Science and Technology for collaboration to conduct this experiment.

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