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\*Corresponding author: Shao-ping Nie,  
State Key Laboratory of Food Science  
and Technology, Nanchang University,  
Nanchang 330047, China  
E-mail: [nie68@sina.com](mailto:nie68@sina.com)

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University, Turkey

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## FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

# Mineral analysis of hulless barley grown in different areas and its $\beta$ -glucan concentrates

Wei-wei Yan<sup>1</sup>, Hao-ying-ye Yao<sup>1</sup>, Shao-ping Nie<sup>1\*</sup> and Ya-jing Li<sup>2</sup>

**Abstract:** Hulless barley (*Hordeum vulgare L. var. nudum Hook. f.*) is reported to have many nutritional functions. In this paper, the contents of K, Mg, Ca, Na, Fe, Zn, Mn, Cu, and Se in 13 cultivars of hulless barley and its  $\beta$ -glucan concentrates were determined. The results showed that there were no marked content differences among cultivars in plant origins, but some elements were enriched in  $\beta$ -glucan concentrates, of which Ca, Fe, Zn, Mn, Cu, and Se are elements related to blood sugar. With the help of  $\beta$ -glucan, this concentrate may be a useful functional food for diabetics.

**Subjects:** Breads; Cereals & Dough; Food Analysis; Nutraceuticals & Functional Foods

**Keywords:** Hulless barley (*Hordeum vulgare L. var. nudum Hook. f.*);  $\beta$ -glucan concentrates; mineral elements; ICP-OES

### 1. Introduction

Hulless barley (*Hordeum vulgare L. var. nudum Hook. f.*) originates from the Qinghai-Tibet Plateau, and it differs from regular hulled barley as it is a barley line with less hull to cover the caryopsis (Yang, Christensen, McKinnon, Beattie, & Yu, 2013a). The plant distributes mainly in Qinghai, Tibet, Sichuan, and Yunnan where the altitudes are above 4,000 m, and it has attracted wide interests around the world (Xiao, Chen, Liang, Lou, & Tang, 2010). Yang et al. have developed four hulless barley cultivars with altered carbohydrate traits and characterized their molecular structure features (Yang, McKinnon, Christensen, Beattie, & Yu, 2014), Zhang et al. investigated the blend DDGS

### ABOUT THE AUTHOR

Shao-ping Nie's research interests are focused on the structure, conformation, and bioactivities of dietary fiber and polysaccharides and the relationship of their structure and bioactivities. He also focuses on developing bioactive ingredients for functional foods and nutraceutical products; developing novel dietary fiber and polysaccharides from natural agricultural products and explores their applications in food, medicinal, and pharmaceutical industries. At the same time, Nie is interested in developing new methodology to determine new harmful chemicals that are produced during the heating process in food industries and studying their mechanisms. The research reported in this paper relates to the field of blood sugar, and we believe that this concentrate may serve as a good dietary supplement for diabetes patients.

### PUBLIC INTEREST STATEMENT

In recent decades, an increasing tendency toward the use of natural products instead of synthetic ones in health management has been observed. It is well known that both mineral elements and  $\beta$ -glucan are associated with the level of blood sugar. In this paper, contents of K, Mg, Ca, Na, Fe, Zn, Mn, Cu, and Se in 13 different cultivars of hulless barley were determined.

In addition, two  $\beta$ -glucan concentrates of different purities were extracted from one same cultivar of hulless barley. We found that Ca, Na, Fe, Zn, Mn, Cu, and Se were enriched in  $\beta$ -glucan concentrates, of which Ca, Fe, Zn, Mn, Cu, and Se are elements related to blood sugar. Therefore, this  $\beta$ -glucan concentrate is beneficial to diabetics, and it is promising to develop natural products as a good dietary supplement for them.

inclusion in hulless barley-based feed and concluded that it could generate great effects on protein metabolic characteristics in hulless barley (Zhang & Yu, 2012), Jha et al. used an *in vitro* model to study the fermentation characteristics of carbohydrate fractions of hulless barley in the pig intestine as it's often incorporated in pig diets (Jha, Bindelle, Rossnagel, Van Kessel, & Leterme, 2011), and Yang et al. have found out that hulless barley cultivars with altered carbohydrate traits significantly improved the truly absorbed protein supply to dairy cattle compared to hulled barley (Yang, Christensen, McKinnon, Beattie, & Yu, 2013b), etc.

Not only hulless barley can be incorporated in animal diets to improve their health, but also it is of great benefit to human beings as well. Since the fibrous hull of the barley kernel tends to limit nutrient uptake, hulless barley offers a potential advantage over hulled barley due to its higher digestibility energy (Thacker, 1999). The grain features high protein, high fiber, high vitamin, low fat, and low sugar when compared with other crops. For instance, the average content of protein in hulless barley is 11.31%, higher than that of wheat, rice, and corn, and its contents of eight essential amino acids are also higher than those of three crops', especially lysine and tryptophan. Its average content of starch is 59.25%, 74–78% of which is amylopectin, in some species even as much as 100%.

Hulless barley also contains various mineral elements that are essential for life growth and development. Mineral elements have many important nutritional functions, they participate in our metabolic and synthetic activities, and their deficiency can lead to undesirable pathological conditions that can be prevented or reversed by adequate supplementation (Zhang & Rui, 2012). In this study, we were concerned about the relationship between mineral elements and diabetes. When it comes to the causes of diabetes, the inorganic element theory believes that metabolic disturbance of carbohydrate, lipid, and protein due to lack of elements related to blood sugar (ERBSs) is the fundamental reason, and K, Mg, Ca, Fe, Zn, Mn, Cu, and Se are regarded as key ERBSs that have great significance in maintaining normal blood sugar level.

At present, there are UV-vis, AAS, and ICP-OES to determine the contents of mineral elements. Among them, ICP-OES is a sensitive, simple, rapid, and accurate method that can simultaneously determine multi-elements in samples with satisfactory results and analyze high matrix samples with high TDS since matrix effects and interferences are not so pronounced for ICP-OES (Šelih, Šala, & Drgan, 2014).

In this study, the contents of nine mineral elements in 13 hulless barley cultivars grown in different areas were determined by ICP-OES; all samples were pretreated by wet digestion method ( $\text{HNO}_3$  and  $\text{HClO}_4$ ).  $\beta$ -glucan concentrates of different purities were extracted from hulless barley; the contents in them were also determined.

**Table 1. Optima 5300DV instrumental operating parameters**

RF power	1,200 W
Plasma	0.8 l/min
Auxiliary	0.2 l/min
Cross-flow nebulizer	15 l/min
Solution uptake rate	1.5 ml/min
Viewing mode	Axial

## 2. Methods and methods

### 2.1. Instrumentation

Mineral analysis was performed on an Optima 5300DV ICP-OES (Perkin-Elmer, USA) with a cross-flow nebulizer; the operating conditions of the instrument are shown in Table 1.

### 2.2. Reagents

Standard solutions of multi-elements used for calibration were purchased from SPEX Company (USA) and serially diluted by HNO<sub>3</sub> (5 + 45) before use, among which K, Ca, Na, and Mg are 1.0 mg/mL, the rest are 0.1 mg/mL. HClO<sub>4</sub>, HNO<sub>3</sub>, and H<sub>2</sub>O<sub>2</sub> were all guaranteed reagent. The water used during the determination was ultra-pure prepared by Milli-Q water purification system (18.2MQ cm), and all glass instruments were soaked in 10% HNO<sub>3</sub> overnight before washing with water.

### 2.3. Preparation of samples

Samples of hulless barley were purchased from several different areas as listed in Table 2, and their contents of  $\beta$ -glucan were determined using a Megazyme kit.

The 13 samples of plant origin were prepared as follows: kernels with hull of each cultivar were crushed in a grinder, passed through 40-mesh filtrate cloth, and powder was mixed thoroughly, collecting them as the samples.

$\beta$ -glucan concentrates of two different purities were extracted through two different procedures from HB6 as it contains the highest  $\beta$ -glucan among 13 cultivars. The two extracts were, respectively, numbered as HB14 and HB15.

HB14 was extracted from hulless barley powder after washing twice with ethanol, ultrasonication, treatment by neutral protease as well as thermostable  $\alpha$ -amylase, and ethanol precipitation. The drying was done using heated air at 60°C in an oven; then, the extract was crushed into powder, and its content of  $\beta$ -glucan was 5.037% as determined using a Megazyme kit.

HB15 was extracted from hulless barley powder after ethanol wash, water wash, treatment by thermostable  $\alpha$ -amylase as well as pancreatin, dialyzation, ethanol precipitation, and re-dissolving in 100% isopropanol. The extract was dried under 40°C heated air and crushed into powder; its content of  $\beta$ -glucan was 63.464%.

**Table 2. Hulless barley grown in different areas and their contents of  $\beta$ -glucan**

Number	Cultivar	Area	Content of $\beta$ -glucan (%)
HB1	Baiqing 1	Haibei Qilian, Qinghai	2.071
HB2	Dulihuang + Kunlun12 + Chaiqing1	Hainan Guinan, Qinghai	2.539
HB3	Little hulless barley	Haixi Dulan, Qinghai	2.544
HB4	Zangqing 320	Rikaze, Tibet	2.152
HB5	Naqu Shannan	Lhasa, Tibet	2.187
HB6	Lhasa 300	Lhasa, Tibet	3.492
HB7	Linshi 300	Tibet	2.178
HB8	Ganqing 1	Gannan, Gansu	2.048
HB9	Long black	Diqing, Yunnan	2.328
HB10	Short white	Diqing, Yunnan	2.347
HB11	Yunqing 2	Diqing, Yunnan	2.530
HB12	Diqing 3	Diqing, Yunnan	2.810
HB13	Diqing	Diqing, Yunnan	2.666

**Table 3. Wavelengths of each mineral element**

Element	K	Na	Ca	Mg	Fe	Mn	Cu	Zn	Se
Wavelength (nm)	766.4	589.5	317.9	285.2	238.2	257.6	327.3	206.2	196.0

#### 2.4. Digestion procedures

Certain amounts of each sample powder were accurately weighed into 100-mL beakers, followed by the addition of little water and 8-mL concentrated HNO<sub>3</sub>. The beakers were capped by watch glasses; the samples were then digested on an electric heating plate. After they cooled down, a little of double-distilled water was added and the samples were heated for further several minutes on an electric heating plate. Finally, the digestion solvent was tipped out into 25-mL-calibrated flasks and ready for determination.

#### 2.5. ICP-OES analysis

Inductively coupled plasma optical emission spectrometry was used for the analysis of mineral elements. ICP-OES allows selecting several characteristic spectral lines simultaneously for determining each element. In general, spectral lines with less interference of coexisting elements' spectral lines, good precision, and high signal–noise ratio are selected. In this study, the wavelengths of each mineral element are shown in Table 3.

The standard stock solution of each element was prepared into a series of mixed standard working solutions, tests on which were carried out under chosen instrumental operating conditions, and calibration curves were drawn automatically by the chemical workstation.

### 3. Results

#### 3.1. Mineral elements in hulless barley

The results showed that hulless barley contains various kinds of mineral elements; their contents are listed in Table 4.

The linear correlation coefficients were between 0.9978 and 0.9999 as in Table 5, showing that method is reliable and can be used for the determination of mineral elements in hulless barley samples.

**Table 4. Contents of mineral elements in 13 hulless barley samples**

mg/kg	K	Na	Ca	Mg	Fe	Mn	Cu	Zn	Se
HB1	4,307.5	97	387	1,384	93.5	16	4.75	21.5	0.06
HB2	3,746	100.5	626	1,169	108.5	20	4.65	13.5	0.05
HB3	4,212	165.4	1,155.5	1,163.5	39.5	16.5	4.6	37	0.06
HB4	4,545.5	252	392	1,150	142.5	16	4.3	18	0.06
HB5	4,592.5	285.5	474.5	1,101	206	16.5	4.25	17.5	0.06
HB6	4,776.5	172	452.5	1,127	94	17.5	6.6	30.5	0.06
HB7	4,974.5	138.5	478.5	1,090	203	19.5	4.05	20	0.08
HB8	4,165.5	182	1,020.5	1,156.5	136	16	3.45	30.5	0.05
HB9	4,763.5	56	480.5	1,144	91.5	12.5	3.7	24.5	0.05
HB10	4,323	47.5	602	1,056.5	44	9.65	2.9	27.5	0.05
HB11	5,523	164.5	532	1,249	63.5	19	5.8	28	0.05
HB12	5,007	221	428.5	1,118	39.5	13.5	5.7	23.5	0.08
HB13	29,125	238.5	2,030	3,892.5	235.5	13.5	<1.0	17	0.06

**Table 5. Linear equations and correlation coefficients of nine mineral elements**

Element	Linear equation	Correlation coefficient <sup>a</sup>
K	$Y = 2911X - 1195.7$	0.9997
Na	$Y = 12410X - 522.1$	0.9999
Ca	$Y = 5518X + 643.6$	0.9999
Mg	$Y = 114200X + 8574.8$	0.9998
Fe	$Y = 31530X - 110.7$	0.9990
Mn	$Y = 250000X + 349.8$	0.9999
Cu	$Y = 102900X + 133.0$	0.9999
Zn	$Y = 28430X + 179.1$	0.9992
Se	$Y = 634.3X + 44.4$	0.9978

<sup>a</sup>Correlation coefficient is a coefficient that means statistical relationships between two variables exist.

**Table 6. Contents of mineral elements in hulless barley (HB6) and its  $\beta$ -glucan concentrates (HB14 and HB15)**

mg/kg	K	Na	Ca	Mg	Fe	Mn	Cu	Zn	Se	Content of $\beta$ -glucan (%)
HB6	4,776.5	172	452.5	1,127	94	17.5	6.6	30.5	0.06	3.492
HB14	1,515.5	167.5	1,229	1,343.5	160	27	5.15	54	0.10	5.037
HB15	164	348	10520	863	27	29	<10	84	0.20	63.464

Though the contents of the same element in all 13 samples are not precisely the same, there are no notable differences among them; we can interpret this phenomenon as being brought out by different cultivars. Referring to previous work done by other researchers, the overall level of mineral contents in hulless barley reported in this paper was consistent with their results. We also found that the content of Fe is obviously higher than other trace elements' among these samples, and according to other researchers, its content sometimes may even be above the content of Na. This may be related with the geological environments and soil conditions for the growth of hulless barley.

We can see that there are many ERBSs in hulless barley, including K, Ca, Mg, Fe, Mn, Cu, Zn, and Se. ERBSs are the constituent parts of many enzymes that are related to glycometabolism of human body; they have significant functions of maintaining normal blood sugar level.

### 3.2. Mineral elements in $\beta$ -glucan concentrates

Hulless barley was rich in  $\beta$ -glucan compared with other crops; when we extracted the concentrates, the mineral elements were highly enriched; their contents are listed in Table 6.

When we looked into the mineral elements in  $\beta$ -glucan concentrates, we found that the process of extraction didn't remove much of them; they were still in the extracts, and content increases were observed in most of them. So were the ERBSs. These increases can be explained by the extracting procedures. The enzymes removed starch, protein, and fat out of hulless barley, but didn't have much direct effect on mineral elements, therefore leading an enrichment of them.

We now illustrate further the details of the two different  $\beta$ -glucan concentrates. Compared with HB14, HB15 contained much higher  $\beta$ -glucan; meanwhile, it was enriched with notably much higher Ca. This could be due to the reason that HB15 was refined pure as well as the conjugation of  $\beta$ -glucan and Ca, which also explained the characteristic of  $\beta$ -glucan being easy to form gels. However, we could see that there were less K and Mg in HB15; HB14 even contained less K than HB6. This might be possibly, on the one hand, because their salts were soluble, and, on the other hand, they were conjugated to soluble polysaccharides and proteins, thus going away with the water through

extraction. Different extracting procedures would lead to different compositions of  $\beta$ -glucan concentrates, and by modifying the process, we could get the desired product with high ERBSs and high  $\beta$ -glucan, which might offer diabetics another possible treatment option.

#### 4. Discussion

Compared with crops like rice and buckwheat, hulless barley can't be considered as a rich source of mineral elements. For instance, raw rice contains 157 mg% Mg (Doesthale, Devara, Rao, & Belavady, 1979), buckwheat contains 5,650 mg/kg K and 2,676 mg/kg Mg (Steadman, Burgoon, Lewis, Edwardson, & Obendorf, 2001), rye flour contains 5,576 mg/kg K, 37 mg/kg Mn, and 31 mg/kg Zn (Ekholm et al., 2007); But according to species and contents of mineral elements, we can still say that hulless barley is a kind of healthy food of high quality. It contains various macroelements as well as microelements, some of which are of considerable amounts. Among them, K, Mg, and Ca can protect the cardiovascular system and Mn is active in forming amino acids (Soetan, Olaiya, & Oyewole, 2010). According to Kashian and Fathivand, nearly 100 specific enzymes depend on Zn for catalytic activity; Zn also participated in certain enzymatic systems required for DNA and RNA syntheses (Kashian & Fathivand, 2015). When it comes to heavy metal, Warman et al. determined the content of Cu in corn grown in Nova Scotia (Warman & Termeer, 2005), and Peris et al. determined the contents of Cu in the edible parts of two types of horticultural crops from 30 agricultural fields in Castellón (Spain) (Peris, Micó, Recatalá, Sánchez, & Sánchez, 2007). Comparing with their results, the content of Cu in hulless barley was found to be quite low. It is known to us that all heavy metals in agricultural soils can affect plants as well as human beings through soil ingestion; therefore, it can be inferred that the clean environment of Qinghai-Tibet Plateau contributed to the low Cu in hulless barley grown there. Besides, from the results, we could see that hulless barley and its  $\beta$ -glucan concentrate contained high Se. Se deficiency and sub-optimality were associated with health disorders including oxidative stress-related conditions (e.g. cardiovascular disease and/or various inflammatory syndromes), thyroid dysfunction, reduced fertility and immune functions, increased susceptibility to viral infection, and increased risk of cancers (Bhatia et al., 2013). Increasing the consumption of hulless barley could be employed as a good strategy to raise Se intake, and its  $\beta$ -glucan concentrates might possibly be developed as a new kind of selenium-rich food (the content of Se above 0.15 mg/kg).

Aside from these benefits, ERBSs were reported to be beneficial to diabetics; they were claimed to be favorable for controlling blood sugar level. The extraction of  $\beta$ -glucan concentrates from hulless barley removed protein, starch, etc., leading to an enrichment of ERBSs, and didn't affect the hypoglycemic activity of ERBSs.

Meanwhile,  $\beta$ -glucan was also favorable to diabetics.  $\beta$ -glucan, a kind of soluble dietary fiber, stayed for relatively a long time in stomach; thus, it wouldn't cause a rapid rise in blood sugar and was suitable for diabetic patients. Its content in hulless barley ranged from 40 to 70 g/kg (Yin et al., 2001).  $\beta$ -glucan could reduce the concentration of plasma LDL cholesterol by promoting the excretion of fecal lipids (Tong et al., 2015). Stimulatory effects of  $\beta$ -glucan on neutrophils, as well as other components of the immune system, have long been recognized (Palic', Andreasen, Herolt, Menzel, & Roth, 2006) as well. Through effectively controlling the level of blood fat and blood sugar, previous researchers concluded that hulless barley containing high  $\beta$ -glucan had favorable functions in diabetes prevention.

Based on the above discussion, we believed that this kind of  $\beta$ -glucan concentrate extracted from hulless barley could serve as a good dietary supplement for people, especially diabetes mellitus patients, through the provision of ERBSs and  $\beta$ -glucan at the same time.

#### 5. Conclusions

The contents of nine mineral elements, of which K, Ca, Mg, Fe, Mn, Cu, Zn, and Se are ERBS, in hulless barley of 13 different cultivars and its two  $\beta$ -glucan concentrates of different purity were determined by ICP-OES and the results were reported in this paper. The results of plant origins showed no marked

differences of contents among cultivars. We can see that hullless barley contains various ERBSs, most of them (Ca, Fe, Zn, Mn, Cu, and Se) were enriched in  $\beta$ -glucan concentrates; the process of extraction doesn't affect the hypoglycemic activity of ERBSs. Meanwhile,  $\beta$ -glucan, a kind of soluble dietary fiber, is also suitable for diabetics. Therefore, we believe that this kind of  $\beta$ -glucan concentrate can serve as a good dietary supplement for diabetes patients through the provision of ERBSs and  $\beta$ -glucan at the same time.

However, a problem has yet to be addressed: that is, we failed to retain some certain ERBSs like K and Mg in the  $\beta$ -glucan concentrates. Therefore, further experiments are in progress to provide deeper insights into the influences of extracting procedures on the contents of mineral elements in  $\beta$ -glucan concentrates.

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#### Competing interests

The authors declared no competing interests.

#### Author details

Wei-wei Yan<sup>1</sup>  
E-mail: [Claire\\_7@126.com](mailto:Claire_7@126.com)  
Hao-ying-ye Yao<sup>1</sup>  
E-mail: [275302875@qq.com](mailto:275302875@qq.com)  
Shao-ping Nie<sup>1</sup>  
E-mail: [nie68@sina.com](mailto:nie68@sina.com)  
Ya-jing Li<sup>2</sup>  
E-mail: [382826038@qq.com](mailto:382826038@qq.com)

<sup>1</sup> State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China.

<sup>2</sup> Qinghai Taer Sacred Valley Agricultural Science and Technology Co Ltd, Xining 810003, China.

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