The chemical composition of barley grain (Hordeum vulgare L.) landraces from the Canary Islands

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Dear Editor,

You can find enclosed herewith a new version of our manuscript (Ms. JFDS-2019-1869.R2) entitled *"The chemical composition of barley grain (Hordeum vulgare* L.) landraces from the Canary Islands".

The manuscript has been carefully corrected and re-written in accordance with the corrections, comments and suggestions suggested by the reviewer.

We have used the red letter to indicate the changes made in the manuscript.

Looking forward to hearing from you, I remain

Yours sincerely

Dr. Elena María Rodríguez Rodríguez

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The chemical composition of barley grain (*Hordeum vulgare* L.) landraces from the Canary Islands

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Abstract: The proximate composition, total phenolics, antioxidant activity, minerals and trace elements were determined in 42 barley landraces in order to highlight their nutritional potential and promote their cultivation. Two-row barley landraces had a higher average content of starch, protein, total phenolic compounds and iron, when compared with six-row ones that presented higher mean ash and fiber concentrations. Additionally, the six-row barley landraces with strongly pigmented grains had lower zinc and manganese and higher protein mean concentrations than the whitish or lightly pigmented barley landraces. Factor analysis and linear discriminant analysis were used to correctly differentiate samples according to type of barley (landrace or commercial) and number of rows. In general, all the parameters varied considerably among the barley landraces analyzed, but some barley landraces could be emphasized according to fiber, minerals and phenolic antioxidants. There were important differences in the mean values in all the chemical parameters according to the island of origin of the barley grains. The application of Linear Discriminant Analysis also was a useful tool to differentiate all the barley landraces with six rows according to the island of origin.

Keywords: barley, landraces, NIR, Canary Island, food composition, mineral and trace elements

Practical Application:

Barley is a versatile cereal that can be used for human and animal feed, brewing and as biodiesel. From the nutritional point of view, barley is rich in starch, protein, dietary fiber and minerals, as well as antioxidant compounds and vitamins. The landraces analyzed in this study have remained intact for the last 900 years, which gives opportunity to genome of these barley landraces to evolve some very specific traits. The physicochemical characterization of these local landraces carried out by us could be very useful as a source of new quality in breeding programs.

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is the most cultivated cereal in Spain with a production of the 5,785,944 t, more than 40% of the total of cereals. It is mainly used to manufacture animal feed (MAPA, 2018), in brewing and in the production of ethanol for biodiesel. The cultivation of two-row barley has increased in Spain and throughout the world due to the presence of new varieties with better brewing qualities and a higher yield compared to six row barley (Callejo-González, 2002). The chemical composition and quality of barley grains is influenced by factors such as genetic characteristics, specific treatments, environmental conditions and agricultural practices (Abdel-Aal & Choo, 2014). This implies that the grain of the same varieties may differ in their chemical composition according to agronomic, climatic, soil conditions and cultivation techniques (Woźniak, Soroka, Stepniowska, & Makarski, 2014).

The first two decades of the 21st century have seen a growing interest in the investigation and development of the use of barley in a wide range of food products (Lee et al., 2008). Analogously to other cereals, the consumption of barley contributes to the intake of starch, protein (deficient in lysine), vitamins B, minerals and trace elements (Kaur, Gill, & Karwasra, 2017). Considerable amounts of proteins, vitamins, minerals and trace elements are commonly found in the husk of the grain, which is closely linked to the grain. Therefore, the flour produced by milling the whole grain has a higher nutritional value and which is commonly associated with more brownish colorations (Márquez-Elías, 2007). In fact, barley has been recognized worldwide including the European Food Standards Agency as having numerous health benefits (EFSA, 2006). This cereal is rich in (1-3) (1-4)-β-D-glucans which are among the most important soluble fibers in human nutrition. These compounds regulate blood glucose levels and reduce the effects of cholesterolemia and blood pressure, and consequently, reduce the risk of cardiovascular diseases. Furthermore, they are considered antioxidant, antibacterial, anti-tumor and anti-inflammatory agents, and play an important role in the immunomodulation and regulation of the human gut flora (Bai et al., 2019). In addition, a high content of fiber in food increases the intestinal transit lowering the exposure of colonocytes to carcinogens and increasing the satiation from a meal which can be useful in weight control (Abdel-Aal et al., 2014). Like the rest of the grains, barley has considerable amounts of phenolic compounds, particularly in the external parts of the grain (cover, testa and aleurone). Flavanols, especially catechin, procyanidins and prodelphinidins, are the main compounds in the free phenolic fraction while phenolic acids, such as ferulic, coumaric and vanillic acids, are major constituents of the bound phenolic fraction (Abdel-Aal et al., 2014).

Geographical isolation allows the genome of traditional and local barley landraces to develop specific traits that could be useful as a source of new quality in breeding programs. Barley landraces from the Canary Islands have been characterized molecularly and morphologically (Hagenblad, Leino, Hernández Afonso, & Afonso Morales, 2019); these landraces were characterized as having a covered grain, rough edges and an absence of pilosity in the basal leaves.

The molecular studies carried out using Single Nucleotide Polymorphism concluded that their genetics have remained intact for the last 900 years (Leino, Hagenblad, Edqvist, & Strese, 2009), which gives an idea of the invaluable legacy that these landraces provide. However, the production of this cereal in the Canary archipelago has markedly decreased in the first two decades of the 21st century, which put these ancient landraces at serious risk, not only for the loss of this genetic heritage but also because of the disappearance of the landscapes associated with this crop (Hagenblad et al., 2019). Knowledge of the nutritional profile of traditional barley landraces of the Canary Islands is a good way to increase the value of this genetic heritage, and thereby encouraging its use and promotion.

The proximate composition, total phenolics and antioxidant activity and minerals and trace elements were determined in traditional barley landraces from the Canary Islands to establish differences not only between the landraces but also with respect to commercial varieties. This characterization will allow the selection of barley landraces with the best nutritional characteristics and the promotion of their cultivation and use. Multivariate analysis was applied to the differentiation of barley landraces according to genetic and agronomic characteristics.

2. MATERIALS AND METHODS

2.1 Description of barley landraces and cultivation

Forty five barley samples were analyzed, 42 were ancient landraces from the Canary Islands, and 3 were commercial ones used as control. Data about island of origin, spike density, number of rows, aleurone layer color, and length of vegetative cycle are shown in Table 1 (Hagenblad et al., 2019). Most of the barley samples were of six rows, only 4 (2 landraces and 2 commercial samples barley) were of two rows.

All the samples were supplied by the Center for the Conservation of Agricultural Biodiversity of Tenerife. All the landraces were grown in the same climatic and soil conditions; in the Araya area (Tenerife Island) (28º21’47” N and 16º 23’38” W), on a farm located at 530 m above sea level. The experimental samples were planted on December 18, 2012, in a flat outdoor plot. The physicochemical characteristics of the irrigation water and soil used are summarized in Supplementary Table S1. The cultivation soil was classified as saline and the irrigation waters had moderate electrical conductivity. These values were considered adequate for the cultivation of this cereal as barley is considered moderately resistant to salinity. Data for accumulated precipitation, temperature, relative moisture and wind speed and, direction were recorded on a monthly basis by a meteorological station located on the same farm. The test cultivation was conducted in a randomized complete block design with three blocks. The sowing density was 350 seeds/m^2 which were sowed manually in 4 rows, 4 m long and 20 cm apart. The plots were fertilized before planting with 300 kg/ha of complex 15-15-15 complex NPK fertilizer. Pest and diseases were chemically controlled following the technical recommendations. Drip irrigation was installed and water was supplied according to the crop needs. Harvesting was performed manually between May and June 2013, when physiological maturity was reached.

2.2 Proximate analysis

A 600 g sample of barley grain was collected from each plot for analysis. A 20 g subsample was cleaned with Milli-Q water and homogenized with an electric grinder (Ika-Werke, Staufen, Germany) to determine phenolic compounds, antioxidant capacity and mineral and trace elements. Moisture, protein, starch, crude fiber and ash were analyzed in the whole grain and in quadruplicate using near-infrared spectroscopy (NIRS) at the "Cereales Archipiélago" (Tenerife, Spain). As quality control of data, moisture, starch, protein and ash were determined in two samples of barley in triplicate, using classical methods (AOAC, 2006) for comparison with the results obtained by NIRS. No significant differences were found in the three analyzed parameters between both methods.

2.3 Total phenolic content and antioxidant activity

Total phenolic content was determined by the Folin-Ciocalteu assay. Briefly, 0.1 g of flour sample was weighed into a polyethylene tube and 6-7 mL of 50% methanol were added. After sonication and centrifugation, the volume was adjusted to 10 mL with 50% methanol. One mL of this acid extract was mixed with 1 mL 50% Folin-Ciocalteu reagent (Sigma-Aldrich Chemical Co., St. Louis, MO). After 5 min, 2 mL 10% Na_2CO_3 solution were added and kept for 10 min in the dark. After centrifugation, the absorbance was measured at 750 nm. Gallic acid was used as a calibration standard and the results were expressed as mg of gallic acid equivalents (GAE)/g dry weight (dw). Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Bondet, Brand-Williams, & Berset, 1997). The DPPH solution (0.1 mM in methanol) was diluted with methanol to obtain an absorbance of 1.00±0.01 at 517 nm. After which, 1 g of flour sample was weighed into a polyethylene tube, and 6-7 mL of 25% methanol was added. After sonication and centrifugation, the volume was adjusted to 10 mL with 25% methanol. After this, 0.15 mL of the extract was mixed and shaken with 1.85 mL of solution of DPPH. The extract was then incubated in the dark for 25 min, and the absorbance was measured at 517 nm. The antioxidant capacity was calculated using a calibration curve prepared with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) within a range of 200-800 μM, and the results were expressed as mg of Trolox equivalents (TE)/g dw.

2.4 Minerals and trace elements

The previously dried and homogenized barley samples were acid-digested using nitric acid, according to the procedure described by Hernández Suárez, Rodríguez Rodríguez, and Díaz Romero (2007). Therefore, 1 g of dried sample was weighed into a digestion tube, and 6 mL of HNO_3 Hiperpur (Panreac, Barcelona, Spain) were added. The mixture was heated until 160°C, finally 1 mL of HCl Hiperpur (Panreac, Barcelona, Spain) was added and the mixture was heated at 160°C/5 min. The acid extract obtained was adjusted to 10 mL. The mineral and trace element analysis was performed on this acid extract in triplicate. The minerals (K, Mg, Ca, Fe, Cu, Zn and Mn) were determined using a Varian SpectraAA 50B (Varian Iberica S. L., Madrid, Spain) atomic absorption spectrometry with flame air-acetylene, equipped with a D_2 lamp background correction system. Phosphorous was determined by the colorimetric method with Vanadate-Molybdate reagent

(BOE, 1995). Briefly, 0.15 mL of the previous acid extract was diluted to 2.5 mL with Milli-Q water. After which 2.5 mL of molybdate-vanadate reagent (Panreac, Barcelona, Spain) were added. The mixture was stirred and left for 10 min to form the colored yellow complex; the absorbance was then measured at 400 nm.

2.5 Statistical analysis

All the statistical analyses were performed using the SPSS version 25.0 for Windows (SPSS Inc., Chicago, USA). Mean values obtained for the variables studied in the different groups were compared by One-Way ANOVA (Duncan's multiple range) assuming there were significant differences among them when the statistical comparison gave $P < 0.05$. A statistical study of correlation between all the quantitative parameters determined was carried out. Factor analysis, using principal components as the method of factor extraction, was used to establish a more simplified view of the relationship among all the analyzed parameters. Linear discriminant analysis was applied to classify the barley samples as homogeneous groups established by the dependent variable.

3. RESULTS AND DISCUSSION

3.1 Proximate composition, total phenolic compounds and antioxidant activity

Results (mean±standard deviation) of the proximate composition, total phenolic compounds and antioxidant activity of the 42 landraces and 3 commercial barley samples are shown in Table 2. The moisture concentrations in the barley landraces ranged between 11.9% and 16.2% and the values were, in general, higher than the data found in the literature (Abdel-Aal et al., 2014). The moisture in barley grains should be low to prevent the growth of microorganisms as well as changes in grain vitreous stability. Six of the 42 barley samples studied had moisture values higher than 15% and therefore had a considerable risk of microbial alteration. Whole cereal grains are considered a good source of mineral and trace elements when compared to refined flours. The content of ash oscillated in a relatively narrow range, 2.4% and 2.9% dw. The ash values found here fall within the range reported by several researchers (Åman, Hesselman, & Tilly, 1985; Holopainen et al., 2014; Ragaee, Abdel-Aal, & Noaman, 2006), but were higher than those contents shown by others (Abdel-Aal et al., 2014; Griffey et al., 2010). Starch is the major carbohydrate in all cereals and contributes significantly to energy intake, while the sugars are found in barley grain at very low concentrations (Gray, Abdel-Alal, Seetharaman, & Kakuda, 2010). The starch contents obtained in the present study are in the range of 52.2-59.2% dw, which was below that of most of the published values (Abdel-Aal et al., 2014; Åman et al., 1985; Griffey et al., 2010; Holopainen et al., 2014). Some investigators reported starch values similar to the ones reported here, such as Ragaee et al. (2006) for naked barley and by Griffey et al. (2010) for husked barley.

Whole cereals make a considerable contribution to fiber intake. In barley, most of the fiber consists of β-glucans and pentoses in variable proportions. The β-glucans are located in the cell wall and aleurone layer, so their proportion is higher in naked grains than in whole grains (Abdel-Aal et al., 2014). The mean concentrations of crude fiber in the 42 barley landraces varied considerably (4.2-8.1% dw), and were higher than those reported by Åman et al. (1985). The proportion of soluble proteins (albumins and globulins) was relatively high (25%) (FEDNA, 2014). The protein concentrations for the barley landraces studied here ranged between 10.8% and 14.0% dw; which was similar to the values reported by many other authors (Abdel-Aal et al., 2014; Åman et al., 1985; FEDNA, 2014; Holopainen et al., 2014; Lahouar et al., 2017). However, the protein content of the barley landraces studied here were higher than other reported data (Bertholdsson, 1999; Griffey et al., 2010) of barley used to make malt in which the protein content should not exceed 11.5% under any environmental condition and all the barley samples analyzed here, except for three, had higher contents than this value. High starch content is another condition to take into account when using barley in malting. The barley landraces analyzed here presented low starch values and, therefore, traditional Canary barley landraces do not appear to meet the standards required for producing industrial beer although they could be used in the artisanal beers which do not require standardized conditions.

Barley grains contain a wide range of phenolic compounds, among which are phenolic acids. These compounds may be the main contributors to the antioxidant activity of barley (Abdel-Aal et al., 2014). In barley, most of these phenolic compounds are bound to other components and, therefore, free phenolics account for a small portion of the total phenolic compounds (Abdel-Aal et al., 2014). Besides, some antioxidant compounds of soluble fiber constituents could inhibit the digestive absorption of minerals. In addition, there is great variability in their concentrations due to differences in genotype as well as environmental conditions (Abdel-Aal et al., 2014). The concentrations of total phenolic compounds and antioxidant activity in the barley samples varied between 117-284 mg GAE/100 g dw and 0.59-0.96 mg TE/g dw, respectively. These values are approximately twice those values found for wheat landraces from the Canary Islands (Hernández Rodríguez, Afonso Morales, Rodríguez-Rodríguez, & Díaz Romero, 2018). The results obtained here for the phenolic compounds were similar to those obtained by Abdel-Aal et al. (2014) and Lahouar et al. (2017) and higher than those reported by Ragaee et al. (2006).

3.2 Minerals and trace elements

Table 3 shows the results regarding minerals and trace elements in all the barley samples. Differences were found among barley samples in the concentrations of most of the minerals. There are several environmental factors affecting the mineral content of the barley grains including cultivation soils, water and fertilizers (chemical, organic and biofertilizers) (Farahani, Caichi, Mazaheri, Afshari, & Savaghebi, 2011). The same environmental conditions were maintained during this study, so the differences were due to genetic characteristics of the barley samples.

Although, barley contains appreciable amounts of minerals, its bioavailabilities are low due to the formation of insoluble complexes with phytates, because they reduce the intestinal absorption of minerals in humans (Sandberg et al., 1999). The phosphorus contents (1829-5179 mg/kg) in the barley landraces varied greatly among the samples analyzed. Most of the phosphorus in cereal grains is in phytate form; a lower bioavailability of minerals such as calcium, iron or zinc could be observed when the phosphorous content in barley is higher. The values found here were within the range described by other authors (Farahani et al., 2011; Woźniak et al., 2014) and lower than those published by Ragaee et al. (2006) and Lahouar et al. (2017). Potassium and magnesium contents were similar to phosphorous, which is within the range described by Woźniak et al. (2014) and lower than those results reported by others (Ragaee et al., 2006). ~~The barley samples had similar magnesium concentrations to those reported by other investigators Woźniak et al. (2014), but lower than the results reported by Ragaee et al. (2006).~~ The calcium concentrations were lower than the other three major minerals, similar to the mean concentrations found in trace elements with relatively high contents, such as iron and zinc. Ragaee et al. (2006) found calcium concentrations above the data found here; however, Kandemir, Tuzen, Sari, and Mendel (2005) found lower calcium concentrations than those in this study. Coated barley has more calcium than naked barley, which could explain some of the differences detected in the literature (Kandemir et al., 2005).

As regards the trace elements, the iron content was the highest. Farahani et al. (2011) suggested that the iron concentration present in the barley grain is influenced by genetic characteristics more than by the environmental effects. The iron concentrations of the barley landraces were similar to those reported by other authors (El-Haramein & Grando, 2008; Farahani et al., 2011; Kandemir et al., 2005), but lower than those found by Ragaee et al. (2006) and Woźniak et al. (2014). ~~The Moroccan barley varieties (El-Haramein et al., 2008) were characterized by their high iron and zinc content.~~ ~~The~~Our results ~~here~~ concerning iron ranged between 33.7 and 68.3 mg/kg dw; only six landraces had concentrations near the 60 mg/kg observed ~~in the above mentioned paper (~~by El-Haramein et al. (2008)~~.~~ in Moroccan barley varieties. High concentrations of zinc were found in barley grains when the crop is under conditions of water stress, so this mineral may be mobilized to the grain (Farahani et al., 2011). These investigators found a significant increase in the zinc, nitrogen and manganese contents in the grain of 27%, 12% and 7%, respectively, in drought stressed barleys. Zinc concentrations varied within a relatively narrow range (38.3-58.1 mg/kg). The mean zinc concentration found by us was lower than that reported by Ragaee et al. (2006) and Farahani et al. (2011) although it resembles the data on covered varieties

(El-Haramein et al., 2008) and is greater than other published values (Kandemir et al., 2005). The barley samples had manganese concentrations ranging between 11.5-19.4 mg/kg dw, which were similar to most of the published data (Kandemir et al., 2005; Ragaee et al., 2006; Woźniak et al., 2014), but lower than those found by Farahani et al. (2011), Copper had the lowest concentrations of all the studied trace elements-which were within the range reported by other authors (Kandemir et al., 2005; Ragaee et al., 2006; Woźniak et al., 2014).

3.3 Comparison of chemical composition between type of barleys and number of rows

The analyzed barley landraces had higher mean ash and fiber concentrations than commercial barley and lower mean starch, protein and total phenolic compounds concentrations than commercial barley. No significant differences were observed in antioxidant activity. Major minerals (phosphorous, potassium and magnesium) in commercial barley varieties had higher mean concentrations than the analyzed barley landraces; however, no significant differences in all mean trace element concentrations were observed between both types of barley samples.

Most (95%) of barley landraces analyzed in the present study had six rows. Considerable differences were found between the two and six-row barley landraces. Raw fiber, Fe, Ca, Mg, Mn and phenolic compounds were the nutritional parameters used to nutritionally evaluate the barley landraces because of the consumption of barley can contribute to their daily intake. Therefore, some landraces (nº 4, 6, 7, 9, 22) could be emphasized since they had relatively high levels of these nutrients. The six-row barley landraces had higher mean ash and fiber concentrations and lower starch, protein and total phenolic compounds than the two-row barley landraces. Furthermore, the fiber data obtained in the commercial barleys, both six-row and two-row, were closer to those of the local two-row barley. The two-row barley landraces (nº 3 and nº 5) had the lowest mean fiber concentrations (4.2% and 4.5%, respectively) and the highest starch concentrations (59.2% and 57.4%, respectively). Some investigators (Åman et al., 1985) found that the starch content of the six-row varieties was higher than that in two-row varieties, which coincides with the results here obtained; however, Holopainen et al. (2014) found the opposite. Therefore, the number of rows and spike density, genetically determined, influenced the proximate chemical composition of the barley landraces. No significant differences in mineral and trace element contents were detected except for iron. The two-row barley landraces had higher mean iron content than the six-row barley landraces.

3.4 Comparison of chemical composition according to spike density, aleurone layer color and island of origin

As regards the six-row barley landraces which were selected for this study, those with a lax spike density had lower mean moisture and fiber concentrations that those with an intermediate spike density (Table 4). No significant differences were found for the rest of the parameters considered. The aleurone layer color did not have a significant influence on ash, protein, starch, crude fiber, total phenolic compounds and antioxidant activity. The mean moisture content in slightly pigmented barley landraces was higher than in those with a whitish or strongly pigmented aleurone layer, and significant differences were found with respect to the latter. Legzdina and Mezaka (2008) found a significant correlation between the β-glucan contents of the crude fiber and the color of the grain and, therefore naked barley, which has a high content of β-glucans, tends to have an association with a light color of the grain.

The island of origin of the landrace grains influenced the production and chemical composition parameters. Significant differences were found according to the island of origin for all the chemical parameters (except total phenolic compounds). The barley landraces from La Palma had the highest moisture levels; and the landraces from the island of Lanzarote had the lowest moisture values. Significant differences were found between barley landraces from the islands of La Palma, Gran Canaria and Tenerife. Barley landraces from La Palma and Gran Canaria Islands presented lower mean ash concentrations than those barley samples from Lanzarote and Fuerteventura. The mean starch content in the landraces from La Palma was higher than those mean contents found in the landraces from Fuerteventura, La Gomera and Lanzarote. The mean fiber content in the barley landraces from Fuerteventura was higher than the mean fiber content observed in barley landraces from Tenerife, Gran Canaria and La Gomera. Variance analysis was used to classify the barley landraces into three groups as a function of protein content. The barley landraces from Gran Canaria and La Gomera had a higher mean protein concentration than those from La Palma, Lanzarote and Fuerteventura. Barley landraces from Tenerife were not statistically different to those from the other islands. The mean antioxidant activity (DPPH) values in the barley landraces from Gran Canaria and Fuerteventura were lower than those obtained in barley landraces from La Palma and La Gomera.

The results of the mineral and trace element contents in six-row barley landraces according to spike density, aleurone layer color and origin island are shown in Table 5. Barley landraces with a lax density of spike had higher mean major mineral contents (P, K, Mg and Ca) and lower mean zinc and manganese concentrations than the six-row landraces with an intermediate spike density. Major mineral concentrations (except phosphorous) were not influenced by the aleurone layer color; however, all the trace elements were affected. Barley landraces with a strongly pigmented aleurone layer presented lower mean phosphorous, iron, copper, zinc and manganese concentrations than those observed in the barley landraces with whitish (except iron) or slightly pigmented aleurone. The island of origin of the landrace grains influenced certain minerals and trace elements such as potassium, calcium, copper and manganese. Barley landraces from Fuerteventura had the lowest mean manganese and copper concentrations, and significant differences were found with the corresponding landraces from other islands, and for copper in those landraces from Tenerife, La Palma and Gran Canaria. Besides, the landraces from Fuerteventura had the highest mean calcium concentration, and significant differences were found in the barley landraces from Tenerife, Gran Canaria and La Gomera; barley landraces from La Palma had a higher mean potassium concentration than the mean value found in the landraces from Lanzarote.

3.5 Correlational study

A statistical study of correlation between all the quantitative parameters analyzed in the barley landraces showed a number of significant correlations. Ash presented inverse correlations with starch (r = -0.727), protein (r = -0.373), and phenolic compounds (r = -0.361), and a positive correlation with fiber (r = 0.861). It is noteworthy that no positive correlations were found between ash and major minerals such as phosphorous, potassium and magnesium. In addition to ash, fiber showed a strong inverse correlation ($P < 0.001$) with starch (r = -0.743) and proteins (r = -0.555), and a moderate correlation ($P < 0.01$) with phenolic compounds. Besides, the starch was weakly correlated ($P < 0.05$) with phenols and copper. The starch-crude fiber correlation suggests that both food components may be synthesized one from the other. Phenolic compounds only correlated (r = 0.438) with phosphorus and antioxidant activity did not show significant correlations. The fact that phenolic compounds were not correlated with antioxidant activity suggests that it must have other antioxidant food components with no phenolic chemical structure contributing significantly to the antioxidant activity of barley. All the trace elements were correlated ($P < 0.001$) with each other except for iron with copper. Besides, the major minerals phosphorous, potassium and magnesium were correlated ($P < 0.001$) with each other; however, the behavior of calcium was different. The common and complex metal interactions occurring in soils, water and plants could influence the correlations observed here.

3.6 Multivariate analysis

Factor analysis was applied to all the samples to establish a more simplified view of the relationship among the analyzed variables. The first five factors, accounting for 76.0% of the total variance, were chosen because their eigenvalues were higher than 1, and therefore, they explain more variance than the original variables. A Varimax rotation was carried out to minimize the number of variables influencing each factor. The first factor that explains the higher percentage of variance (25.0%) is strongly and inversely associated with fiber and ash and is positively associated with starch. The second factor (19.4% of variance) is related with manganese and zinc and, to a lesser extent, with iron. The third factor (12.7% of variance) is associated with magnesium and phosphorus and the fourth and fifth factors with moisture and antioxidant capacity (DPPH), respectively. The score plots of all the barley samples for the first and third factors (Figure 1), show that the barley landraces were well separated from the commercial varieties. Besides, commercial barley and barley landraces were differentiated according to the number of rows. Stepwise linear discriminant analysis (LDA) was applied on quantitative variables to differentiate the barley samples according to the number of rows and landrace and commercial barleys. A complete classification (100%; 91.1% after cross-validation) of the four groups of barley samples was prepared by selecting the following variables: ash, fiber, proteins, potassium and iron.

A subsequent LDA introducing all the chemical variables was conducted to classify the six-row barley landraces according to spike density, aleurone layer color and island of origin. A correct classification of 90.0% was observed according to the island of origin using all the chemical variables; although this classification decreased by 37.5% after cross-validation. All the barley landraces from La Palma, La Gomera, Lanzarote and Fuerteventura were correctly classified. Only three of the barley samples of Tenerife Island were erroneously classified as being from La Gomera (1) and Gran Canaria (2); and one from Gran Canaria was included as being from Tenerife. Figure 2 shows the representation of all the six-row barley landraces of the LDA results according to the island of origin. One can see that the six groups of barley landraces associated to the considered islands were reasonably differentiated.

4. CONCLUSION

~~Canary barley landraces are a good source of phyto-nutrients and other bioactive compounds such as proteins, soluble fibers, antioxidants such as phenolic compounds, minerals and trace elements.~~ From a technological point of view, the barley landraces analyzed here are not suitable for malting because of their high protein and fiber contents. Notable differences in the proximate composition, TP and antioxidant activity and mineral and trace element contents were observed among the analyzed barley landraces. Canary barley landraces are a good source of phyto-nutrients and other bioactive compounds such as proteins, soluble fibers, antioxidants such as phenolic compounds, minerals and trace elements. The six-row barley landraces nº 7 and 22 could be selected as possible candidates in biofortification programs to increase the nutrient density in barleys due to their high levels of raw fiber, iron, calcium, and phenolic compounds. Many differences were found in the chemical composition-among the barley samples according to the type of barley (commercial and landrace), the number of rows and island of origin of the barley grains. No phenolic chemical compounds significantly contribute to the antioxidant activity of landrace barleys, which is deduced due to the lack of correlation between phenolic compounds and antioxidant activity. Many correlations between trace elements and major minerals were observed which could be associated with the metal interactions occurring in the soils, water and plants. The application of multivariate analysis on the chemical composition is a useful tool to differentiate and characterize landrace barleys. Thus, factor and discriminant analyses can be applied to differentiate the analyzed barley samples according to the type of barley (commercial and landrace), number of rows and island of origin.

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AUTHOR CONTRIBUTIONS

D.A.M. and D.R.M. supplied the barley samples and characterized them morphologically. M.P.C. performed the research, P.D.E. helped with NIRS data. M.P.C., B.R.G., CD.R. and E.M.R.R. designed the research, analyzed the data and wrote the paper.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abdel-Aal, E. M., & Choo, T.-M. (2014). Differences in compositional properties of a hulless barley cultivar grown in 23 environments in eastern Canada. *Canadian Journal of Plant Science*, *94*, 807-815. https://doi.org/10.4141/cjps2013-301

Åman, P., Hesselman, K., & Tilly, A.-C. (1985). The variation in chemical composition of Swedish barleys. *Journal of Cereal Science, 3*, 73-77. https://doi.org/10.1016/S0733-5210(85)80035-7

AOAC International. (2006). *Official methods of analysis of AOAC International* (18th Ed). Horwitz, W., Latimer Jr., G.W. (Eds.). Gaithersburg, MD: AOAC International.

Bai, J., Ren, Y., Li, Y., Fan, M., Qian, H., Wang, L., Wu, G., Zhang, H., Qi, X., Xu, M., & Rao, Z. (2019). Physiological functionalities and mechanisms of β-glucans. *Trends in Food Science and Technology, 88*, 57-66. https://doi.org/10.1016/j.tifs.2019.03.023

Bertholdsson, N. O. (1999). Characterization of malting barley cultivars with more or less stable grain protein content under varying environmental conditions. *European Journal of Agronomy, 10*, 1-8. https://doi.org/10.1016/S1161-0301(98)00043-4

BOE. (1995). *RD 2257/1994, de 25 de noviembre, por el que se aprueba los métodos oficiales de análisis de piensos o alimentos para animales y sus primeras materias*. BOE Nº 52 de 2 de marzo de 1995, 7161-7235.

Bondet, V., Brand-Williams, W., & Berset, C. (1997). Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. *LWT - Food Science and Technology, 30*, 609-615. https://doi.org/10.1006/fstl.1997.0240

Callejo-González, M. J. (2002). *Industrias de cereales y derivados* (pp. 337). Madrid: Mundiprensa.

EFSA. (2006). EFSA panel on dietetic products, nutrition and allergies (NDA): scientific opinion on the substantiation of a health claim related to “oat beta-glucan” and lowering blood cholesterol and reduced risk of (coronary) heart disease pursuant to article 14 of regulation (EC) No 1924/2006. *EFSA Journal, 8*, 1885.

El-Haramein, F. J., & Grando, S. (2008). Determination of iron and zinc content in food barley. In S. Ceccarelli and S. Grando (Eds.), *Proceedings of the 10th International Barley Genetics Symposium* (pp. 603-606). Alexandria, Egypt: ICARDA.

Farahani, S. M., Caichi, M. R., Mazaheri, D., Afshari, R. T., & Savaghebi, G. H. (2011). Barley grain mineral analysis as affected by different fertilizing systems and by drought stress. *Journal of Agricultural, Science and Technology, 13*, 315-326.

FEDNA, 2014. Cebada dos carreras nacional 11,3% PB. Fundación Española para el Desarrollo de la Nutrición Animal. http://www.fundacionfedna.org/node/495, Accessed date: 1 April 2017.

Gray, D., Abdel-Alal, E. S. M., Seetharaman, K., & Kakuda, Y. (2010) Differences in viscosity and textural properties of selected barley cultivars as influenced by pearling and cooking. *Food Chemistry, 120*, 402-409. https://doi:10.1016/j.foodchem.2009.10.020

Griffey, C., Brooks, W., Kurantz, M., Thomason, W., Taylor, F., Obert, D., Moreau, R., Flores, R., Sohn, M., & Hicks, K. (2010). Grain composition of Virginia winter barley and implications for use in feed, food, and biofuels production. *Journal of Cereal Science, 51*, 41-49. https://doi.org/10.1016/j.jcs.2009.09.004

Hagenblad, J., Leino, M. W., Hernández Afonso, G., & Afonso Morales, D. (2019). Morphological and genetic characterization of barley (*Hordeum vulgare* L.) landraces in the Canary Islands. *Genetic Resources and Crop Evolution, 66*, 465-480. https://doi.org/10.1007/s10722-018-0726-2

Hernández Rodríguez, L., Afonso Morales, D., Rodríguez-Rodríguez, E. M., & Díaz Romero, C. (2018). Physicochemical characteristics and proximate composition of wheat cultivars from the Canary Islands. *Acta Alimentaria, 47*, 122-129. https://doi.org/10.1556/066.2018.47.1.15

Hernández Suárez, M., Rodríguez Rodríguez, E. M., & Díaz Romero, C. (2007). Mineral and trace element concentrations in cultivars of tomatoes. *Food Chemistry, 104*, 489-499. https://doi.org/10.1016/j.foodchem.2006.11.072

Holopainen, U. R. M., Pihlava, J.-M., Serenius, M., Hietaniemi, V., Wilhelmson, A., Poutanen, K., & Lehtinen, P. (2014). Milling, water uptake, and modification properties of different barley (*Hordeum vulgare* L.) Lots in relation to grain composition and structure. *Journal of Agricultural and Food Chemistry, 62*, 8875-8882. https://doi.org/10.1021/jf500857e

Kandemir, N., Tuzen, M., Sari, H., & Mendel, D. (2005). An increase of the mineral contents of barley grain by genotype, planting time and seed size. *Asian Journal of Chemistry, 17*, 481-489.

Kaur, H., Gill, B. S., Karwasra, B. L. (2017). Physicochemical, functional, pasting and antioxidant properties of flours from different cereals: A comparative evaluation. *International Journal of Advanced Biotechnology and Research, 8*, 283-292.

Lahouar, L., Ghrairi, F., El Arem, A., Medimagh, S., El Felah, M., Salemd, H.B., & Achour, L. (2017). Biochemical composition and nutritional evaluation of barley rihane (*Hordeum vulgare* L.). *African Journal of Traditional, Complementary and Alternative Medicines, 14,* 310-317. https://doi.org/10.21010/ajtcam.v14i1.33

Lee, M. J., Kim, Y. K., Kim, M. J., Hyun, J. N., Jonson, G., Kim, J. G., & Kim, H. S. (2008). Nutritional characteristics and properties of barley grown in Korea. In S. Ceccarelli and S. Grando (Eds.), *Proceedings of the 10th International Barley Genetics Symposium* (pp. 607-612). Alexandria, Egypt: ICARDA.

Legzdina, L., Mezaka, I. (2008). Progress of the hulless barley breeding program. In S. Ceccarelli and S. Grando (Eds.), *Proceedings of the 10th International Barley Genetics Symposium* (pp. 61-67). Alexandria, Egypt: ICARDA.

Leino, M. W., Hagenblad, J., Edqvist, J., & Strese, E.-M. K. (2009). DNA preservation and utility of a historic seed collection. *Seed Science Research, 19*, 125-135. https://doi.org/10.1017/S0960258509990055

MAPA. (2018). Ministerio de Agricultura, Pesca y Alimentación. Retrieved May 6, 2018 from: http://www.mapa.gob.es.

Márquez-Elías, A. K. (2007*). Elaboración y evaluación de un producto de panificación con harina de cebada. Instituto de Ciencias Básicas e Ingeniería*. Mexico: Universidad Autónoma del Estado de Hidalgo.

Ragaee, S., Abdel-Aal, E.-S. M., & Noaman, M. (2006). Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry, 98*, 32-38. https://doi.org/10.1016/j.foodchem.2005.04.039

Sandberg, A. S., Brune, M., Carlsson, N. G., Hallberg, L., Skoglund, E., & Rossander-Hulthén, L. (1999). Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. *The American journal of clinical nutrition, 70*, 240-246. https://doi.org/10.1093/ajcn.70.2.240

Woźniak A., Soroka, M., Stepniowska, A., & Makarski, B. (2014). Chemical composition of spring barley (*Hordeum vulgare* L.) grain cultivated in various tillage systems. *Journal of Elementology, 19*, 597-606. https://doi.org/10.5601/jelem.2014.19.2.438

Table 1-Description of barley landraces.

N	Island	Rows	Spike density	Aleurone color	Lenght of vegetative cycle
1	Tenerife	6	Lax	Green	159
2	Tenerife	6	Lax	Green	143
3	Tenerife	2	Intermediate	White	158
4	Gran Canaria	6	Lax	Green	159
5	Tenerife	2	Intermediate	White	145
6	Gran Canaria	6	Lax	White	152
7	Lanzarote	6	Lax	Green	162
8	La Palma	6	Lax	Green	153
9	Gran Canaria	6	Lax	Green	152
10	La Gomera	6	Lax	Green	141
11	Tenerife	6	Lax	Blue	138
12	Tenerife	6	Lax	Green	153
13	Gran Canaria	6	Lax	Green	150
14	Gran Canaria	6	Lax	Green	161
15	Tenerife	6	Lax	Green	138
16	Tenerife	6	Lax	Green	155
17	Fuerteventura	6	Lax	Green	154
18	La Palma	6	Lax	Green	152
19	La Gomera	6	Lax	Blue	150
20	Gran Canaria	6	Lax	Green	157
21	Gran Canaria	6	Lax	Green	159
22	Tenerife	6	Lax	Green	161
23	La Gomera	6	Lax	Blue	150
24	La Palma	6	Lax	Green	147
25	Gran Canaria	6	Lax	Green	161
26	Tenerife	6	Lax	Green	161
27	Tenerife	6	Lax	Green	162
28	Gran Canaria	6	Lax	Green	155
29	Gran Canaria	6	Lax	Green	151
30	Gran Canaria	6	Intermediate	Green	162
31	Gran Canaria	6	Lax	Green	156
32	Gran Canaria	6	Lax	Green	114
33	Gran Canaria	6	Lax	White	157
34	Gran Canaria	6	Lax	Blue	155
35	Lanzarote	6	Lax	Blue	140
36	Tenerife	6	Lax	Blue	150
37	Gran Canaria	6	Lax	Green	150
38	Tenerife	6	Lax	Blue	156
39	Gran Canaria	6	Lax	Blue	144
40	Tenerife	6	Lax	White	151
41	Tenerife	6	Lax	Green	150
42	Tenerife	6	Lax	Green	151
43	Control	6	Intermediate	White	184
44	Control	2	Intermediate	White	147
45	Control	2	Intermediate	White	148

Table 2-Results (mean±standard deviation) of the major chemical components, total phenolic compounds and antioxidant activity of the barley samples.

Number	Moisture (%)	Ash (%)	Starch (%)	Crude Fiber (%)	Protein (%)	Total phenols (mg GAE/100 g)	DPPH (mg TE/100 g)
1 [a]	13.9±0.15	2.84±0.04	54.1±0.41	7.44±0.14	12.0±0.13	210±19	0.95±0.02
2 [a]	13.1±0.16	2.76±0.05	53.8±0.41	7.08±0.13	12.6±0.23	259±19	0.89±0.05
3 [b]	13.7±0.40	2.43±0.07	59.2±0.68	4.21±0.12	13.3±0.07	202±18	0.86±0.07
4 [a]	13.5±0.20	2.78±0.08	54.3±0.68	7.66±0.25	11.7±0.15	220±15	0.86±0.09
5 [b]	13.3±0.09	2.63±0.10	58.3±0.67	4.94±0.28	12.8±0.12	284±15	0.78±0.04
6 [a]	13.7±0.25	2.74±0.05	54.6±0.66	7.14±0.22	12.8±0.23	206±23	0.75±0.07
7 [a]	12.5±0.20	2.87±0.05	53.8±0.41	7.86±0.16	11.4±0.18	197±7.3	0.88±0.06
8 [a]	13.3±0.16	2.72±0.03	54.4±0.14	7.68±0.16	11.4±0.26	163±6.5	0.91±0.06
9 [a]	12.9±0.18	2.75±0.06	54.6±0.30	7.53±0.23	11.9±0.26	247±17	0.76±0.06
10 [a]	13.3±0.19	2.82±0.03	52.6±0.42	7.67±0.08	12.5±0.30	181±9.7	0.93±0.05
11 [a]	12.1±0.14	2.80±0.01	52.4±0.26	7.50±0.16	13.0±0.38	170±15	0.86±0.08
12 [a]	13.7±0.11	2.80±0.03	53.4±0.15	7.84±0.06	12.0±0.22	168±2.7	0.92±0.02
13 [a]	13.3±0.29	2.74±0.02	53.4±0.40	7.42±0.23	12.5±0.14	259±16	0.90±0.06
14 [a]	11.9±0.15	2.81±0.05	53.0±0.26	7.48±0.07	12.7±0.33	147±10	0.88±0.02
15 [a]	12.1±0.29	2.83±0.04	52.2±0.31	7.34±0.10	13.1±0.24	155±6.4	0.76±0.06
16 [a]	13.2±0.13	2.80±0.03	53.5±0.27	7.58±0.14	11.6±0.27	167±15	0.90±0.05
17 [a]	12.9±0.17	2.84±0.03	53.1±0.40	7.99±0.03	11.6±0.19	193±3.8	0.77±0.01
18 [a]	14.6±0.36	2.76±0.03	54.5±0.34	7.57±0.13	11.6±0.34	221±23	0.96±0.03
19 [a]	12.5±0.17	2.65±0.02	54.8±0.61	5.83±0.23	13.7±0.19	200±17	0.91±0.03
20 [a]	12.0±0.09	2.73±0.05	53.7±0.29	6.96±0.22	13.4±0.09	148±12	0.87±0.07
21 [a]	13.9±0.23	2.91±0.05	52.8±0.23	7.73±0.15	12.9±0.28	153±11	0.84±0.06
22 [a]	12.8±0.30	2.84±0.06	53.3±0.81	7.69±0.22	12.3±0.07	205±23	0.86±0.02
23 [a]	14.1±0.09	2.87±0.02	53.2±0.26	7.97±0.16	11.9±0.13	178±4.8	0.90±0.02
24 [a]	15.9±0.19	2.83±0.05	54.4±0.40	7.87±0.07	11.7±0.22	117±6.4	0.94±0.03
25 [a]	16.2±0.22	2.76±0.03	55.1±0.12	7.16±0.13	12.2±0.04	134±10	0.85±0.02
26 [a]	14.3±0.15	2.82±0.04	54.9±0.65	7.67±0.12	11.6±0.08	168±6.3	0.79±0.05
27 [a]	15.5±0.21	2.82±0.02	55.3±0.21	7.80±0.16	10.8±0.17	145±3.4	0.66±0.01
28 [a]	12.6±0.13	2.79±0.06	52.2±0.41	7.44±0.19	13.7±0.27	179±17	0.62±0.05
29 [a]	13.5±0.13	2.75±0.02	53.6±0.34	6.89±0.33	13.2±0.08	194±6.4	0.59±0.02
30 [a]	14.6±0.23	2.83±0.04	53.5±0.11	8.05±0.10	11.7±0.16	172±11	0.82±0.01
31 [a]	12.9±0.14	2.74±0.04	54.0±0.30	7.56±0.03	11.5±0.21	181±13	0.75±0.02
32 [a]	16.0±0.55	2.74±0.03	54.6±0.50	7.03±0.08	12.9±0.24	174±31	0.82±0.02
33 [a]	13.9±0.37	2.79±0.02	54.1±0.46	7.46±0.27	12.1±0.31	205±5.2	0.82±0.02
34 [a]	12.4±0.18	2.74±0.07	52.3±0.43	7.35±0.17	13.9±0.11	168±9.0	0.83±0.02
35 [a]	12.3±0.18	2.79±0.04	53.2±0.50	7.69±0.18	12.0±0.15	175±4.1	0.85±0.06
36 [a]	13.4±10.18	2.79±0.07	53.6±0.51	7.69±0.21	11.8±0.33	170±15	0.90±0.05
37 [a]	15.5±0.06	2.73±0.08	54.1±0.69	6.98±0.13	13.5±0.18	193±19	0.85±0.03
38 [a]	15.3±0.22	2.78±0.07	54.3±0.60	7.22±0.18	12.7±0.33	199±18	0.91±0.04
39 [a]	13.1±0.23	2.71±0.05	53.3±0.57	7.37±0.25	12.4±0.24	181±1.1	0.84±0.03
40 [a]	12.8±0.08	2.85±0.05	52.9±0.81	7.37±0.17	12.6±0.27	154±0.6	0.87±0.03
41 [a]	13.7±0.09	2.79±0.04	53.6±0.42	7.53±0.17	12.1±0.03	166±3.4	0.86±0.05
42 [a]	13.6±0.12	2.74±0.07	53.8±0.82	6.44±0.44	14.0±0.46	220±9.4	0.88±0.04
Total	13.6±1.11	2.78±0.08	54.0±1.34	7.30±0.75	12.4±0.77	186±35	0.84±0.08
43 [1,a]	11.9±0.25	2.70±0.05	57.4±0.40	4.54±0.20	13.4±0.45	204±19	0.90±0.06
44 [1,b]	12.6±0.18	2.70±0.02	54.8±0.28	6.07±0.23	13.2±0.10	192±11	0.87±0.05
45 [1,b]	13.4±0.17	2.55±0.09	57.9±0.32	5.01±0.11	12.3±0.16	218±19	0.85±0.05

Data are shown in dry weight. [1] Commercial barley; [a] Six rows; [b] Two rows.

Table 3-Results (mg/kg dw, mean±standard deviation) of the mineral and trace element concentrations of the landraces and commercial barley samples.

Number	P	K	Ca	Mg	Fe	Cu	Zn	Mn
1 [a]	4707±237	3751±190	396±10.2	1360±81.0	51.6±3.41	6.76±0.13	52.8±2.41	17.4±0.46
2 [a]	4576±199	4055±161	454±29.6	1461±38.0	49.3±0.88	7.10±0.12	51.6±0.29	15.9±0.17
3 [b]	4054±179	4014±10.7	384±23.2	1371±31.4	50.8±2.64	7.81±0.18	50.0±0.25	15.8±0.66
4 [a]	4687±209	4052±169	423±7.41	1506±37.7	49.0±4.08	7.65±0.15	51.0±1.18	16.8±0.63
5 [b]	4611±301	3854±158	426±14.7	1385±44.4	62.8±1.79	6.24±0.48	49.4±3.37	17.5±1.07
6 [a]	4959±250	4383±10.0	441±21.0	1369±64.6	52.1±3.21	7.51±0.30	54.1±1.74	17.3±0.59
7 [a]	4295±301	3592±102	504±17.6	1422±19.1	54.3±2.83	6.98±0.11	58.1±1.15	17.9±0.32
8 [a]	4528±198	3808±206	447±15.0	1395±34.7	45.3±1.28	6.26±0.16	50.9±1.91	13.7±0.55
9 [a]	4446±303	4098±43.3	366±15.8	1356±54.2	51.4±4.64	8.04±0.20	55.2±1.39	17.6±0.56
10 [a]	3906±96.0	3545±199	482±10.0	1372±26.7	43.6±2.81	4.87±0.15	40.6±1.58	13.6±0.35
11 [a]	4510±236	4157±162	425±10.1	1381±19.2	47.5±2.20	5.96±0.61	47.5±1.25	14.0±0.43
12 [a]	3899±118	3891±247	444±28.1	1287±70.5	41.8±1.10	5.85±0.58	43.0±1.22	14.2±0.57
13 [a]	4352±147	4231±148	394±12.7	1329±45.5	44.1±0.56	6.55±0.04	49.2±0.29	15.6±0.18
14 [a]	3941±131	4305±140	451±28.3	1376±41.2	49.1±1.02	5.74±0.18	46.5±1.31	14.2±0.37
15 [a]	4447±288	4020±85.7	480±32.3	1367±69.2	46.0±1.41	6.27±0.10	48.6±0.52	15.1±0.27
16 [a]	4144±400	3862±112	425±3.91	1306±25.0	42.1±1.96	6.39±0.07	42.2±1.58	14.1±0.32
17 [a]	4204±182	3645±105	498±10.6	1399±45.3	41.1±0.74	5.60±0.09	42.6±0.77	12.4±0.24
18 [a]	4413±271	4191±77.6	503±46.2	1414±98.5	43.3±2.90	6.70±0.26	43.3±2.11	15.0±0.62
19 [a]	3556±263	3620±35.9	519±9.42	1434±58.8	48.8±3.44	6.02±0.36	47.8±3.61	13.9±0.73
20 [a]	2774±107	4309±104	483±13.2	1351±29.3	64.7±5.30	6.31±0.14	47.0±1.25	15.7±0.34
21 [a]	3525±256	4435±17.6	480±31.4	1351±134	68.4±1.74	6.95±0.26	54.5±1.58	19.2±0.81
22 [a]	3666±115	3376±261	538±18.4	1296±81.2	53.8±6.05	6.48±0.37	47.3±2.81	18.1±0.56
23 [a]	3428±368	3436±342	363±35.3	1172±84.0	57.1±4.09	8.44±0.99	54.1±6.82	17.8±2.56
24 [a]	3640±333	4120±256	512±8.15	1424±31.7	49.6±0.16	8.44±0.22	53.9±0.66	17.9±0.50
25 [a]	3421±277	3786±342	472±2.69	1273±37.2	60.5±0.61	8.40±0.53	51.8±1.91	17.1±0.67
26 [a]	3909±302	3684±180	453±30.8	1441±54.7	34.6±2.64	7.11±0.18	46.7±1.57	13.3±0.38
27 [a]	4165±319	3731±119	413±32.9	1581±133	44.5±2.40	8.27±0.37	55.7±3.20	14.4±0.97
28 [a]	3371±120	3580±20.8	435±23.5	1162±33.1	59.6±2.66	6.37±0.45	50.3±3.12	17.3±1.26
29 [a]	3476±132	3833±154	454±39.8	1218±88.9	41.1±2.36	7.78±0.22	51.1±1.59	16.8±0.63
30 [a]	2073±60.2	3000±156	384±22.5	1107±85.0	48.3±1.24	6.67±0.29	56.5±1.77	19.4±0.70
31 [a]	4565±432	3729±252	367±34.7	1265±174	40.4±2.78	7.61±0.06	48.6±1.47	13.8±0.55
32 [a]	4311±410	3640±176	421±15.4	1129±23.0	37.3±1.77	6.89±0.11	48.2±1.84	14.5±0.45
33 [a]	5179±29.4	3624±189	401±19.9	1169±110	35.6±3.07	6.82±0.15	45.7±0.66	15.6±0.21
34 [a]	3151±60.1	3656±123	456±33.1	1296±32.3	41.5±3.60	6.60±0.38	44.4±3.10	15.1±0.55
35 [a]	3196±110	3430±307	471±24.0	1221±80.1	36.8±2.08	5.32±0.19	42.2±2.67	11.5±0.29
36 [a]	3057±164	3435±67.1	364±24.8	1036±18.2	34.8±0.41	6.04±0.13	42.6±0.72	12.9±0.37
37 [a]	4273±107	4126±138	437±25.7	1336±137	37.7±2.83	6.40±0.25	46.5±1.70	13.9±0.70
38 [a]	3443±34.4	3815±37.6	523±35.8	1296±59.7	45.3±1.87	6.19±0.25	41.6±1.36	13.7±0.17
39 [a]	3197±305	3849±65.8	429±20.0	1251±110	33.7±0.73	5.44±0.08	38.3±0.07	13.2±0.07
40 [a]	1829±68.9	3220±36.6	484±19.9	1196±83.4	39.0±0.85	6.77±0.13	51.0±1.49	15.9±0.52
41 [a]	2233±115	3667±267	393±4.34	1101±93.1	60.7±3.18	7.57±0.19	54.3±1.00	18.4±0.46
42 [a]	3063±96.1	3652±269	417±5.15	1147±8.13	45.5±0.24	5.80±0.22	49.9±3.43	14.5±1.13
Total	3838±764	3814±319	443±46.8	1312±118	47.2±8.63	6.74±0.89	48.7±4.80	15.5±1.95
43 [1,a]	4609±274	4534±93.5	380±23.7	1463±40.4	30.7±1.32	6.89±0.25	48.3±1.61	14.7±0.82
44 [1,b]	4433±172	4747±203	435±17.0	1477±68.6	47.9±2.95	7.06±0.18	51.8±1.94	15.2±0.53
45 [1,b]	4718±355	4449±265	381±26.0	1446±120	51.0±1.42	5.87±0.27	49.5±1.69	14.8±0.76

[1] Commercial barley; [a] Six rows; [b] Two rows.

Table 4-Contents of the major chemical compounds, total phenolic compounds and antioxidant activity in six-rows landrace barleys according to spike density, aleurone layer color and precedence island.

	Moisture (%)	Ash (%)	Starch (%)	Crude Fiber (%)	Protein (%)	Total phenols (mg GAE/100 g)	DPPH (mg TE/100 g)
Spike density							
Intermediate	14.6±0.2 ^{b}	2.83±0.04 ^{a}	53.5±0.1 ^{a}	8.05±0.1 ^{b}	11.7±0.2 ^{a}	172±11 ^{a}	0.82±0.01 ^{a}
Lax	13.5±1.1 ^{a}	2.79±0.07 ^{a}	53.7±0.9 ^{a}	7.43±0.4 ^{a}	12.4±0.8 ^{a}	184±34 ^{a}	0.84±0.09 ^{a}
Aleurone layer color							
White	13.4±0.5 ^{bc}	2.79±0.06 ^{a}	53.9±1.0 ^{a}	7.32±0.3 ^{a}	12.5±0.4 ^{a}	188±29 ^{a}	0.81±0.06 ^{a}
Green	13.7±1.2 ^{c}	2.79±0.06 ^{a}	53.8±0.9 ^{a}	7.49±0.4 ^{a}	12.3±0.8 ^{a}	184±37 ^{a}	0.84±0.09 ^{a}
Blue	13.2±1.0 ^{ab}	2.77±0.08 ^{a}	53.4±0.9 ^{a}	7.35±0.6 ^{a}	12.6±0.8 ^{a}	180±16 ^{a}	0.88±0.05 ^{a}
Island							
Tenerife	13.6±0.9 ^{b}	2.81±0.05 ^{ab}	53.7±0.9 ^{ab}	7.44±0.4 ^{ab}	12.4±0.8 ^{ab}	183±34 ^{a}	0.86±0.08 ^{bc}
La Palma	14.6±1.1 ^{c}	2.77±0.06 ^{a}	54.5±0.3 ^{b}	7.71±0.2 ^{bc}	11.6±0.3 ^{a}	167±47 ^{a}	0.94±0.04 ^{c}
Gran Canaria	13.6±1.3 ^{b}	2.77±0.07 ^{a}	53.7±0.9 ^{ab}	7.38±0.4 ^{ab}	12.6±0.7 ^{b}	186±36 ^{a}	0.80±0.09 ^{ab}
Lanzarote	12.4±0.2 ^{a}	2.83±0.06 ^{b}	53.5±0.5 ^{a}	7.77±0.2 ^{bc}	11.7±0.3 ^{a}	186±13 ^{a}	0.87±0.06 ^{bc}
La Gomera	13.1±0.8 ^{ab}	2.79±0.09 ^{ab}	53.3±1.1 ^{a}	7.29±0.9 ^{a}	12.7±0.7 ^{b}	182±16 ^{a}	0.90±0.05 ^{c}
Fuerteventura	12.9±0.2 ^{ab}	2.84±0.03 ^{b}	53.1±0.4 ^{a}	7.99±0.1 ^{c}	11.6±0.2 ^{a}	193±3.8 ^{a}	0.77±0.01 ^{a}

Data are shown in dry weight. Results in the same vertical column with the same superscript were not significantly different.

Table 5-Contents (mg/kg dw) of the mineral and trace elements in six-rows landrace barleys according to spike density, aleurone layer color and precedence island.

	P	K	Ca	Mg	Fe	Cu	Zn	Mn
Spike density								
Intermediate	2072±60 ^{a}	2998±156 ^{a}	384±23 ^{a}	1107±85 ^{a}	48.2±1.2 ^{a}	6.67±0.3 ^{a}	56.4±1.8 ^{b}	19.4±0.7 ^{b}
Lax	3857±747 ^{b}	3829±332 ^{b}	447±50 ^{b}	1314±131 ^{b}	46.7±8.8 ^{a}	6.72±0.9 ^{a}	48.5±5.1 ^{a}	15.4±2.0 ^{a}
Aleurone layer color								
White	3988±1627 ^{b}	3741±519 ^{a}	442±40 ^{a}	1244±121 ^{a}	42.2±7.9 ^{a}	7.03±0.4 ^{b}	50.2±3.8 ^{b}	16.3±0.9 ^{b}
Green	3897±711 ^{b}	3852±343 ^{a}	446±48 ^{a}	1329±132 ^{a}	48.2±8.6 ^{b}	6.82±0.9 ^{ab}	49.6±4.8 ^{b}	15.8±2.0 ^{b}
Blue	3442±480 ^{a}	3674±287 ^{a}	444±62 ^{a}	1261±130 ^{a}	43.2±8.0 ^{a}	6.25±1.0 ^{a}	44.8±5.4 ^{a}	14.0±1.9 ^{a}
Island								
Tenerife	3626±876 ^{a}	3705±280 ^{ab}	445±53 ^{ab}	1298±159 ^{a}	45.3±7.5 ^{a}	6.66±0.7 ^{b}	48.3±5.0 ^{a}	15.2±1.8 ^{b}
La Palma	4194±481 ^{a}	4040±244 ^{b}	488±39 ^{bc}	1411±56 ^{a}	46.1±3.2 ^{a}	7.13±1.0 ^{b}	49.4±5.0 ^{a}	15.5±1.9 ^{b}
Gran Canaria	3864±837 ^{a}	3919±388 ^{ab}	429±41 ^{a}	1285±124 ^{a}	47.9±11 ^{a}	6.93±0.8 ^{b}	49.3±4.7 ^{a}	16.0±1.9 ^{b}
Lanzarote	3747±636 ^{a}	3512±223 ^{a}	488±26 ^{bc}	1322±122 ^{a}	45.5±9.9 ^{a}	6.15±0.9 ^{ab}	50.1±8.9 ^{a}	14.7±3.5 ^{b}
La Gomera	3850±490 ^{a}	3690±342 ^{ab}	447±64 ^{ab}	1340±114 ^{a}	49.3±5.9 ^{a}	6.32±1.5 ^{ab}	47.5±6.1 ^{a}	14.8±2.1 ^{b}
Fuerteventura	4203±182 ^{a}	3644±105 ^{ab}	498±11 ^{c}	1399±45 ^{a}	41.1±0.7 ^{a}	5.60±0.1 ^{a}	42.6±0.8 ^{a}	12.4±0.2 ^{a}

Results in the same vertical column with the same superscript were not significantly different.

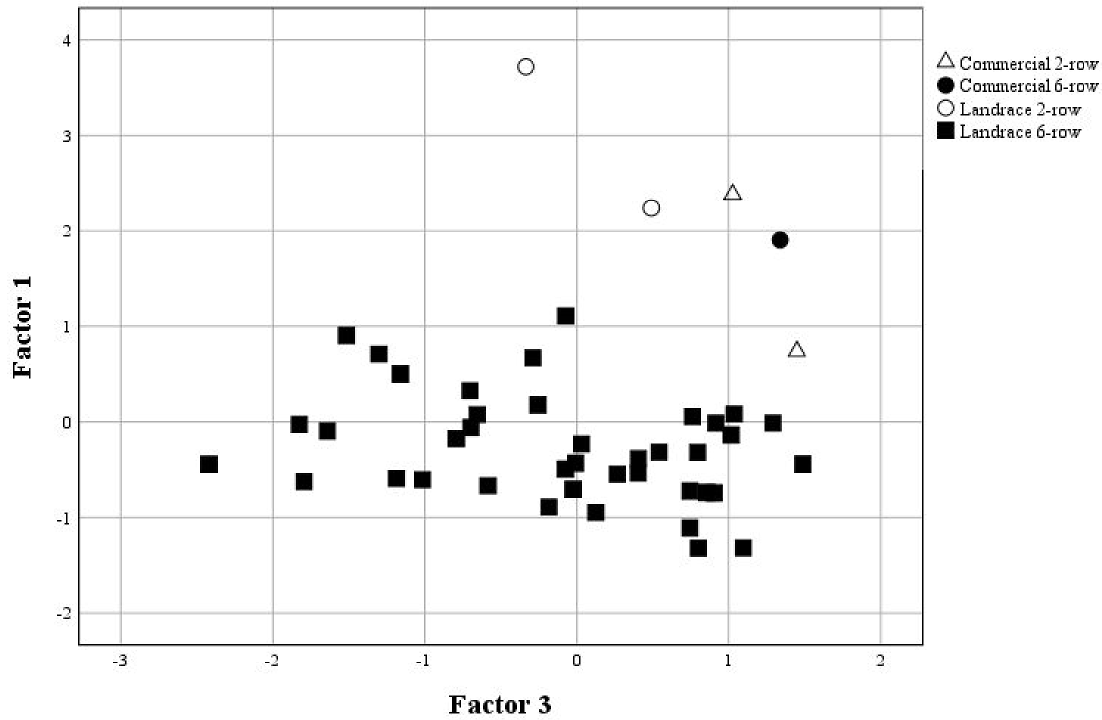

Figure 1-Scores of the barley samples on axes representing the first and third factors differentiating the samples according to the commercial and landrace barleys and number-row.

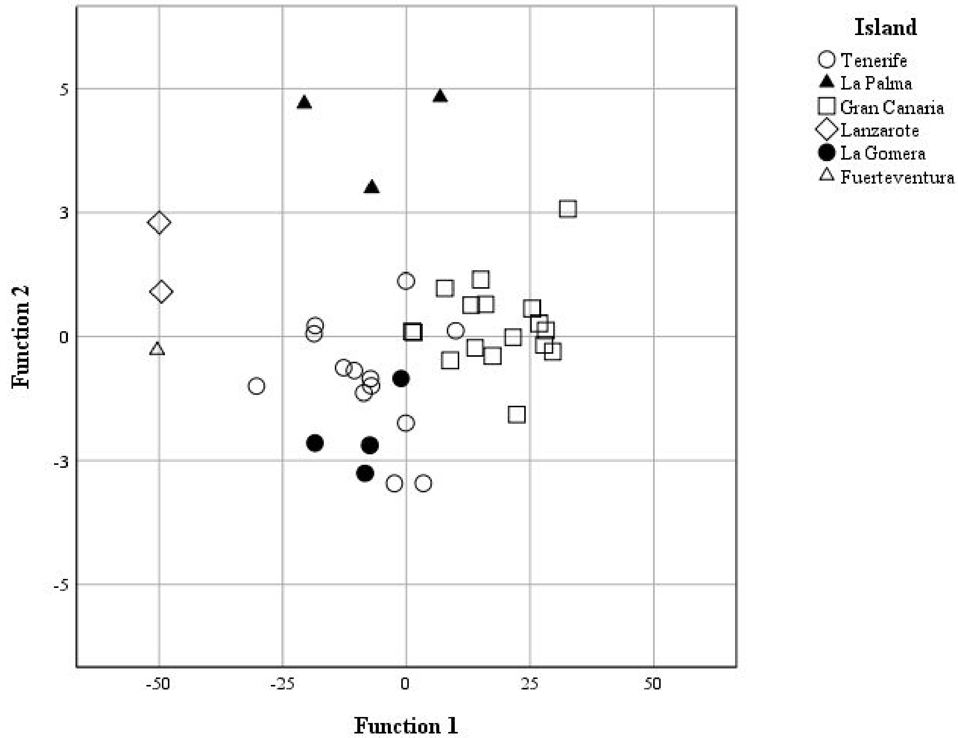

Figure 2-Scatter diagram on the axes representing the two discriminant functions differentiating the landrace barley of six-row according to the origin island.

Supplementary Table 1-Climatological data during the experiment.

Month	T	T_M	T_m	P	RH	V_o	V_{Max}
December-12	16.0	20.7	12.8	3.4	62.2	1.5	3.2
January-13	15.1	20.5	11.5	0.1	62.7	1.4	3.0
February-13	14.9	19.7	11.6	44.7	63.8	1.5	3.7
March-13	15.8	20.3	12.5	90.9	77.6	1.5	3.8
April-13	17.9	23.0	13.8	7.0	60.2	1.7	3.8
May-13	17.5	23.1	13.4	1.0	59.9	1.7	3.9
June-13	17.5	23.2	13.1	0	63.5	1.6	3.1
Total average	16.3	21.5	12.6	147*	64.2	1.6	3.5

* Cumulative precipitation. T: average temperature (ºC); T_M: daily average maximum temperature (ºC); T_m: daily average minimum temperature (ºC); P: precipitation (mm); RH: average relative humidity (%); V_o: average wind speed (m/s); V_{Max}: daily average maximum wind speed (m/s)

Irrigation analysis

pH	EC (25 ºC) (mS/cm)	Carbonates (meq/L)	Bicarbonates (meq/L)	Chlorides (meq/L)	Na (meq/L)	K (meq/L)	Ca (meq/L)	Mg (meq/L)	Equilibrium pH	Adjusted SAR
8.5	0.95	0.4	5.9	3.9	7.4	0.6	0.5	2.8	7.3	12.1

EC: Electrical conductivity

Soil analysis

Organic matter (%)	Phosphorus (mg/kg)	Cations extracted with ammonium acetate (% C.E.C.)				Estimated C.E.C. (meq/100 g)	pH saturated paste	EC saturated extract (mS/cm)	Percent of base saturation (%)
		Na	K	Ca	Mg				
4.7	132	14.1	23.9	45.1	16.9	27.7	7.0	4.18	40.8

Manuscript: "Chemical composition of barley grain (*Hordeum vulgare* L.) landraces from Canary Islands", JFDS-2019-1869.R2

We have used the red letter to indicate the changes made in the manuscript.

Responses to the reviewer' comments:

Reviewer: 1

Manuscript is improved, underlining the importance of present results.

Line 236-242: There are unnecessary repetitions, so this part could be shortened into one sentence.

Ok, both phrases were united to remove the repetition.

Line 250-253: Repetition again, please, exclude this part, irrespective that different elements were considered.

Ok, a sentence was eliminated and other was adequately modified.

Line 338-339: It is strange that there was present correlation between ash content and any of tested mineral elements, indicating that no particular element dominates, as an ash constituent. As it was previously mentioned, this variability gives great opportunity to examined landraces to be part of breeding programs for improved nutritional quality.

The mentioned sentence emphasized that "no positive correlations were found between ash and major minerals (P, K and Mg)". We agree: It is strange that no particular element dominates in the ash content.

We have proposed two barley landraces according to the nutritional content (including raw fiber, minerals, and phenolic compounds) to be part of breeding programs for improved nutritional quality.

Line 343: Please correct – one from the other.

Ok, the phrase was corrected.

Line 381-383: Please, replace this sentence to line 390. Here, it is out of the context.

Ok, the phrase was changed.