Nutritional quality characterization of a set of durum wheat landraces from Iran and Mexico

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Abstract

Wheat grain represents an important source of bioactive components that are associated with different health benefits, notably: dietary fibers, micronutrients and phytochemicals. However, despite the importance of these components, limited data are available on their content and composition, especially in durum wheat. In this study, 82 durum wheat landraces from Iran and Mexico were analyzed for arabinoxylan, iron, zinc, phytate and phenolic acids content. In general, wide variation was identified among landraces for these traits. Specifically, values ranging from 1.4-2.7% and 0.3-1.0% were detected for total and water-extractable arabinoxylans, respectively, in flour In the case of micronutrients varied from 32.7 to 46.1 mg/kg (iron) and from 46.7 to 83.9 mg/kg (zinc) in grain. Phytate, a major component limiting micronutrient bioavailability, varied from 0.7-1.1% in whole-meal flour and the resulting phytate:iron and phytate:zinc molar ratios were 13.7-26.6 and 11.6-21.9, respectively, with more than 70% of the landraces exhibiting a relatively high Zn bioavailability. Seven phenolic acids were identified, with a variation in total phenolic acid concentration ranging from 279 µg/g to 845 µg/g in whole-meal flour. Overall, these results indicate that the landraces analyzed here could serve as useful genetic resources for the improvement of wheat nutritional quality in breeding programs.

Keywords: durum wheat; arabinoxylans; phenolic compounds; micronutrients; phytic acid

1. Introduction

Durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) Husn.) is the most cultivated and economically important tetraploid wheat species, adapted to Mediterranean climates and regions. It is the primary raw material used in the production of diverse foods, including pastas, couscous, and flat breads (Peña-Bautista, Hernandez-Espinosa, Jones, Guzman, & Braun, 2017). Some of these products have a global importance and provide an important amount of calories and proteins to human diets. In addition, durum wheat represents an important source of a wide range of bioactive compounds associated with different health benefits. These bioactive components are mainly contained in the grain bran and germ tissues, and include, among others, dietary fiber, phenolic acids and micronutrients.

Dietary fiber is the group of carbohydrates which are resistant to digestion and absorption in the small intestine and thus reaches the large intestine or colon where they promote gut health. They constitute between 11.5% and 15.5% of the dry wheat grain (Shewry & Hey, 2015) and are mainly composed by non-starch polysaccharides (NSP). Arabinoxylans (AX) constitutes 70% of the total NSP content (De Santis et al., 2018). Based on their solubility, AX can be divided into two fractions: water-unextractable arabinoxylans (WU-AX) and water-extractable arabinoxylans (WE-AX). Both fractions of AX have been associated with positive human health effects. Specifically, the WU-AX have been associated with reduced transit time and increased fecal bulk, greater frequency of defecation and with binding and excretion of carcinogens. In contrast, WE-AX are more readily fermentable in the colon and have been associated with prebiotic activity, stimulating the growth of the intestinal microorganisms (Moore, Park, & Tsuda, 1998). Interestingly, from the analysis conducted on different wheat varieties (Finnie, Bettge, & Morris, 2006; Gebruers et al., 2008; Ciccoritti, Scalfati, Cammerata, & Sgrulletta, 2011), the content of AX can be highly variable

and highly influenced by the genotype, suggesting that improvement through selecting for these polysaccharides, is possible.

Wheat grain is also a good source of micronutrients such as selenium (Se), iron (Fe) and zinc (Zn), which are mainly located in the aleurone layer and the embryo and being scarce in the grain endosperm These minerals are fundamental not only for the plant health but also for human well-being. Specifically, Fe is an essential component of hemoglobin and is fundamental to insure normal cellular and metabolism function and correct growth and development of the body. Similarly, Zn is fundamental for the immune system, protein synthesis and cell division and, among the others, to support the normal growth and development during pregnancy, childhood, and adolescence (Jurowski, Szewczyk, Nowak, & Piekoszewski, 2014). A minimum daily intake of Fe and Zn is necessary to maintain a steady state of these micronutrients in the body. For these reasons, breeding biofortified wheat with enhanced micronutrient concentrations has emerged as a long-term, sustainable solution for micronutrient deficiency and, in the past years, several studies have been conducted to identify and breed germplasm with increased Zn and Fe micronutrient content (Velu et al., 2019). Significant amounts of anti-nutrients are present in the aleurone layer, such as phytic acid which chelates with micronutrients such as Fe and Zn reducing their bioavailability (Eagling, Wawer, Shewry, Zhao, & Fairweather-Tait, 2014). For this reason, when breeding for enhanced micronutrient quantity, it is important also to select lines with lower phytic acid content (Magallanes-Lopez et al., 2017; Ficco et al., 2009).

Phenolic acids represent the most abundant metabolites of whole wheat grain and the most common form of phenolic compounds (Fernandez-Orozco, Li, Harflett, Shewry, & Ward, 2010; Li, Shewry, & Ward, 2008). They occur in three forms: free, conjugated to low molecular weight molecules (e.g. sugars, sterols), or bound to cell wall polysaccharides such

as arabinoxylans (Martini, et al., 2015; Fernandez-Orozco et al., 2010; Li et al., 2008). The bound form is the most abundant in wheat, representing over 80% of total phenolic acids. Recently, phenolic acids have received much attention, mainly due to their antioxidant, anti-inflammatory and anti-carcinogenic properties (Laddomada et al., 2015). For this reason, several studies have been conducted to analyze the variability in phenolic acids content across different wheat varieties (Laddomada et al., 2015; Li et al., 2008; Martini et al., 2015; Pasqualone, Delvecchio, Mangini, Taranto, & Blanco, 2014) revealing a wide range, albeit with low heritability. A similar range of variation in the phenolic compound content was also identified among a set of durum wheat varieties (Laddomada et al., 2017) where the genotypic effect on the phenotypic variation seemed to be greater, suggesting the possibility to improve the wheat grain phenolic acid content through breeding.

Wheat landraces represent a potentially useful genetic resource for the improvement of modern wheat varieties and significant efforts have been made to identify landraces with unique phenotypes to include in breeding programs (Alvarez & Guzman, 2018; Velu et al., 2019). Few studies have thoroughly analyzed wheat landraces for nutritional quality, or the studies were limited to a small number of accessions. Therefore, a wider screening of wheat landraces may be required to more fully exploit the genetic potential for wheat nutritional quality improvement. The International Maize and Wheat Improvement Center (CIMMYT) Germplasm Bank holds approximately 48,600 bread and durum wheat landrace accessions. Among these, 6,947 accessions are from Iran and 14,211 are from Mexico. In our study, a subset of these Iranian and Mexican durum wheat landraces, was analyzed for arabinoxylan, phenolic acids, phytic acid and micronutrient content. The results of this study should help to identify genetic resources that may be exploited in wheat improvement programs to develop cultivars with enhanced health benefits.

2. Materials and Methods

2.1 Plant material

Thirty-nine Iranian and forty-three Mexican durum wheat landraces obtained from CIMMYT Wheat Germplasm Bank (Texcoco, Edo. de Mexico, Mexico) (Electronic Supplementary Table 1) were evaluated in this study. These landraces were grown in Mexicali, Mexico, during the 2015–2016 cropping season. All genotypes were planted without replication in December 2015 and harvested in the beginning of June 2016. Plots were managed following standard agronomic practices for the site.

2.2 Grain parameters

Thousand kernel weight (TKW) (g) and test weight (TW) (kg/hL) were estimated with SeedCount digital image system SC5000 (Next Instruments, Australia). Grain protein (GPRO, 12.5% moisture basis) was measured using near-infrared spectroscopy (DA 7200 NIR, Perten Instruments, Sweden), validating its calibration with method 46-12, 44-15A and 44-01 according to the AACC (AACC, 2010). Grain samples previously conditioned to 16% moisture were milled into flour (farina) using a Brabender Quadrumat Senior mill (C.W. Brabender OHG, Germany).

2.3 Arabinoxylan (AX) determination

The content of arabinoxylans was determined using the colorimetric method reported by Douglas et al. (1981) with some modifications from Finnie et al. (2006). Specifically, 125 mg of flour were suspended in 25 mL of H_2O in a 50 mL Falcon tube. The tubes were vortexed and immediately after 0.5 mL of the sample suspension were transferred in a new

stoppered reaction tubewith 0.5 mL of distilled water. This tube was used to determine TOT-AX content. Then, the Falcon tube was mixed for 30 min in a laboratory rocker. Two mL of this suspension were transferred to a 2 mL Eppendorf type tube, centrifuged at 2,500 x g for 10 min and then 0.5 mL of the supernatant were collected in a stoppered reaction tube with 0.5 mL of distilled water. This fraction was used for the WE-AX analysis. Both the fractions obtained for the TOT-AX and WE-AX analysis were then thoroughly mixed with 5 mL (5:1) of freshly prepared extraction solution and placed in boiling water for 25 min to hydrolyze sugars. During the 25 min the tubes were mixed every 8 min in vortex. One liter of extraction solution contains 932 mL of acetic acid, 17 ml of concentrated hydrochloric acid, 42.4 mL of phloroglucinol 20% (w/v) in ethanol, and 8.5 mL of glucose (1.75% w/v). After, the tubes were placed in ice-cold water and kept cool for about 2 min. Finally, 300 µL of the TOT-AX and WE-AX solutions were placed into a 96-well microplate in duplicate and their absorbance at 552 and 510 nm was measured using an Epoch microplate spectrophotometer (BioTek, Winooski, VT, U.S.A.). The AX content was determined based on a calibration curve generated with known quantities of xylose and using the following equation:

$$\text{Arabinoxylan content (mg xylose/g of sample)} = [(\Delta A_{552\text{-}510}) / (\text{xylose equivalent intercept, mg})] * 200$$

2.4 Zinc, iron, phytic acid and molar ratios determination

Grain iron (FeC, mg/kg) and zinc (ZnC, mg/kg) concentrations were determined by using a bench-top, non-destructive, energy dispersive X-ray fluorescence spectrometry (EDXRF) instrument (Oxford Instruments, UK). For phytic acid determination the protocol described by Magallanes-López et al., (2017) was used. To calculate the molar ratios of phytic acid:iron (Phy:Fe) and phytic acid:zinc (Phy:Zn), the contents of phytic acid, Fe and

179 Zn, were converted into moles by dividing the concentrations by their respective molar mass
180 and atomic weight (660.04, 55.85 and 65.4 g mol^{-1}, respectively).
181
182 *2.5 Phenolic acids determination*
183 Total phenolic acids (comprising soluble and insoluble fraction) were extracted from
184 250 mg whole-meal semolina and analyzed by HPLC analysis following the procedures
185 shown in Laddomada et al., (2017). Whole-meal samples were de-lipidated twice by adding
186 5 mL hexane per time, stirring for 15 min, and centrifuging at 6000 x g for 10 min. Internal
187 standard solution (10 mL of 1.5 mg/mL 3,5-dichloro-4-hydroxybenzoic acid in 80:20
188 methanol/water) was added to the residue prior to hydrolysis with 2 M NaOH for 2 h, with
189 continuous shaking, at 4 °C, in the dark.
190 After hydrolysis, the supernatant was acidified to pH 2 with 12 M HCl (2.4 mL) and
191 submitted to extraction with ethyl acetate for three times. The ethyl acetate extracts were
192 combined, dried under nitrogen flux, redissolved in 100 µL of 80:20 methanol/water and
193 qualiquantitatively analyzed using an Agilent 1100 Series HPLC-DAD system (Agilent
194 Technologies, Santa Clara, CA, USA) equipped with a reversed phase C18 (2) Luna
195 column (Phenomenex, Torrance, CA, USA) (5 mm, 250 x 4.6 mm) at a column temperature
196 of 30 °C. A mobile phase consisting of acetonitrile (A) and 10 mL/L water solution of
197 H3PO4 (B) was utilized for the following elution program: isocratic elution, 100% B, 0-30
198 min; linear gradient from 100% B to 85% B, 30-55 min; linear gradient from 85% B to
199 50% B, 55-80 min; linear gradient from 50% B to 30% B, 80-82 min; and post time, 10 min
200 before the next injection. The flow rate of the mobile phase was 1 mL min-1, and the
201 injection volume was 20 µL. The column temperature was maintained at 30 °C. Peaks were
202 identified by comparing their retention times and UV-Vis spectra to those of authentic
203 phenolic standards: p-hydroxybenzoic acid, vanillic acid, syringic acid, p-coumaric acid,
204 sinapic acid and ferulic acid (Sigma-Aldrich, Gillingham, UK). All phenolic acids were
205 quantified via a ratio to the internal standard (3,5-dichloro-4-hydroxybenzoic acid) added to
206 every sample and using calibration curves of phenolic acid standards.
207 **3. Results**
208 *3.1 General kernel characteristics and total and water-extractable arabinoxylans*

Table 1 shows the averages and ranges of different kernel characteristics and bioactive compounds analyzed of the durum landraces. Wide variation in TW, TKW and GPRO was identified among and within the two sets of landraces. Regarding arabinoxylan content, the landraces from Iran exhibited a slightly higher content of TOT-AX (1.9 g/100g) compared to the landraces from Mexico (1.8 g/100g), but the same WE-AX mean content (0.6 g/100g). In both populations, the majority of the cultivars showed a TOT-AX content ranging from 1.60 to 2.25 g/100g and a WE-AX concentration between 0.50 to 0.89 g/100g (Fig. 1). Among the landraces analyzed, the Iranian accession CWI56833 possessed the greatest content of both TOT-AX (2.7%) and WE-AX (1%) across the two populations, representing the best source of arabinoxylans (Supplementary Table 1).

3.2 Micronutrients and phytic acid content

Among the Iranian and Mexican landraces, similar Fe content was identified. Greater differences were identified between the two populations for ZnC with the cultivars originating from Mexico exhibiting a ZnC ranging between 50.7-83.9 mg/kg and a mean ZnC of 64.1 mg/kg compared to the Iranian landraces, which exhibited an average ZnC of 58.4 mg/kg, with a range of 46.7 to 68.1 mg/kg. Similarly to FeC, the phytic acid concentration did not vary much between the two sets of landraces and even if a greater phytic acid variation was identified among the Mexican landraces, both populations exhibited a similar average phytic acid content of 0.8% and 0.9% for the Iranian and Mexican landraces, respectively (Table 1).

In order to estimate the potential bioavailability of both Fe and Zn in the two sets of landraces, the molar ratio between phytic acid and Fe (Phy:Fe) and between phytic acid and Zn (Phy:Zn) was calculated. In general, lower Phy:Fe values were identified among the

Iranian landraces (average 18.8) where more than 20 cultivars exhibited a Phy:Fe value lower than 20 (Fig.1). The Mexican landraces exhibited lower values of Phy:Zn (average 14.2) (Table 1) with more than 25 cultivars exhibiting Phy:Zn values lower than 15 (Fig.1).

The Iranian landrace CWI73342 represented the best source of Fe exhibiting a relatively high FeC (46.1 mg/kg) coupled with a low concentration of phytic acid (0.7%) and a moderate bioavailability (Phy:Fe = 13.7). In contrast, the Mexican landrace CWI52026 represented the best source of Zn, 83.9 mg/kg and a moderately high ZnC bioavailability, as indicated by the Phy:Zn value of 11.9 (Supplementary Table 1).

3.3 Phenolic acids

Seven phenolic acids were identified, namely: p-hydroxybenzoic acid, syringic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid and sinapic acid. As reported in table 1, the landraces from Iran possessed in general greater concentrations of phenolic acids, with the exception of vanillic acid which was, on average, more abundant among the Mexican landraces. Ferulic acid was the most abundant phenolic acid among both the Iranian and Mexican landraces, contributing to 83.9% and 86.9% of total phenolic acids, respectively, followed by sinapic and *p*-coumaric acids.

Between the two populations, the Iranian accessions CWI56690 and CWI72018 contained the greatest amount of total phenolic acids (845.4 and 828.51 µg/g, respectively) even though variation in the concentration of each single phenolic acid were identified between these two landraces (Supplementary Table 1).

3.4 Pearson correlation coefficients

In order to identify possible relationships present among the analyzed phenotypes, a pairwise comparison between all the traits was performed separately for all the landraces (Table 2).

In general, TOT-AX and WE-AX content appeared to be negatively correlated with TW (r = -0.24 and -0.36, respectively). However, no significant association between TOT-AX or WE-AX and either TKW or GPRO was identified.

Regarding the micronutrient content, both Fe and Zn did not appear to be associated with either TW or TKW but highly positive associations were identified between these two micronutrients and GPRO. Similarly, the concentration of phytic acid was significantly associated with GPRO but it was also negatively associated with TKW. A positive correlation between FeC and ZnC (r = 0.49) was identified indicating that the mechanisms regulating the micronutrient accumulation (i.e micronutrients uptake by the roots, translocation of micronutrients to the grain, etc.) are similar for both iron and zinc. However, increasing quantities of Zn were also positively associated with phytic acid content, one of the main component limiting micronutrient bioavailability suggesting that phytic acid and this micronutrient concentration are, to a certain extent, dependent from each other.

Overall, total and individual phenolic acid concentration did not appear to be influenced by either TW, TKW or GPRO. However, significant correlations were also identified between total phenolic acids and each individual phenolic acid and, in general, significant positive correlations were also identified between each individual phenolic acid. Significant positive correlations were also identified between the total phenolic acids content, *p*-coumaric acid, ferulic acid and sinapic acid, and arabinoxylans (both TOT and WE-AX) (r from 0.36 to 0.21). These same phenolic acids, with the exception of *p*-coumaric acid, were

279 also significantly negatively associated with phytic acid content and with Phy:Fe molar ratio
280 (*r* from -0.37 to -0.24).
281
282 **4. Discussion**
283 Wheat landraces represent a potentially useful genetic resource for the improvement
284 of modern wheat varieties. As a matter of fact, in the past few years there has been renewed
285 interest in wheat landraces as they have been proposed to be rich sources of bioactive
286 components and hence suitable for the production of high value food products with enhanced
287 health benefits. However, limited data are available on the contents and composition of
288 bioactive components in durum wheat landraces and therefore, a wider screening of this type
289 of genetic material is needed in order to identify germplasm useful for the improvement of
290 wheat nutritional quality. In our study, the variability of different bioactive components in
291 durum wheat grain were investigated in 39 Iranian and 43 Mexican durum wheat landraces.
292 In general, the variation for TOT-AX found in this study (performed with durum
293 wheat flour) was greater than the one reported by De Santis et al., (2018) in semolina (1.4-
294 1.8 g/100g) but similar to the results reported by Gebruers et al., (2008) in bread wheat flour
295 (1.7-2.3%; 1.3-2.7% for TOT-AX and WE-AX, respectively). However, as expected, the
296 observed TOT-AX values were still lower than the values reported in whole meal (4.5-4.8%
297 reported by Ciccoritti et al., 2011, and 3.3-4.6 g/100g De Santis et al., 2018). Regarding the
298 WE-AX, the content found was comparable with the WE-AX content previously reported for
299 semolina and flour, and slightly lower than the WE-AX content reported in whole meal
300 (Gebruers et al., 2008; Ciccoritti et al., 2011; Marcotuli et al., 2016; De Santis et al., 2018).
301 Significant negative correlations were identified between either TOT-AX or WE-AX
302 and TW (*r* = -0.24 and -0.36, respectively), indicating that in the analyzed populations the

303 content of arabinoxylans is partially influenced by grain filling and by the different bran to
304 endosperm ratio, as also suggested by Shewry and Hey (2015). For example, the Iranian
305 landrace CWI57009 showed high values for TOT-AX (2.21%) and WE-AX (0.8%) but the
306 lowest TW (71.4 kg/hL), which is not desirable from the breeding point of view. However,
307 some exceptions were also found, such as the Mexican landrace CWI52333 which presented
308 high values of AX (2.2% of TOT-AX and 0.73% in WE-AX) and TW and TKW values above
309 the average (76.7 kg/hL and 53.1 g, respectively). Similarly, two Iranian landraces
310 (CWI57719 and CWI57563) exhibited high TOT-AX (2.3 and 2.2%, respectively) and WE-
311 AX values (0.81 and 0.71%, respectively), associated with good grain characteristics
312 (Supplementary Table 1). In these three cultivars, the higher AX content does not appear to
313 be determined by a higher bran to endosperm ratio but rather by an intrinsic higher
314 concentration of AX in the endosperm indicating that these landraces could be exploited to
315 improve the dietary fibers content in flour.

316 When analyzing the results obtained for the micronutrient contents, the FeC found in
317 both sets of landraces was similar to the one reported by Magallanes-Lopez et al., (2017) in
318 reduced irrigation environment. ZnC was higher than the one reported in previous studies
319 (Velu et al. 2019; Magallanes-Lopez et al., 2017; Ficco et al., 2009), especially when
320 compared to the results obtained from the Mexican landraces, where values up to 80 mg/kg
321 were found. As reported in previous studies (Velu et al. 2016, 2019; Magallanes-Lopez et
322 al., 2017; Ficco et al., 2009), a strong positive correlation between FeC and ZnC was
323 identified in landraces analyzed here, corroborating the possibility to simultaneously improve
324 the concentration of both Fe or Zn. Among the analyzed landraces, the Mexican accession
325 CWI52059 exhibited higher than average concentrations of both micronutrients (43.3 mg/kg
326 of Fe and 78.1 mg/kg of Zn) coupled with TW of 77.2 kg/hL and relatively high TKW (47.5

g). Similarly, the Iranian accession CWI71580 presented values of 42.4 mg/kg of FeC and 64.3 mg/kg of ZnC, showing larger grain than the average. These accessions showing high micronutrient concentrations and good grain morphological characteristics could be effectively used in a breeding program to improve the micronutrient concentration in wheat grain.

In order to improve the intake of micronutrients it is fundamental to know their relative bioavailability. Phytic acid is the major component limiting the absorption of micronutrients and, for this reason, one way to estimate their bioavailability is by calculating the molar ratio between phytic acid and the micronutrients. Hurrell & Egli (2010) suggested that the optimal Phy:Fe molar ratio should be < 1 or preferably < 0.4, in order to significantly improve iron absorption, whereas Frontela, Scarino, Ferruzza, Ros, & Martínez (2009) suggested that Phy:Fe values ranging from 1.4 to 3.8 are indicative of a reduced iron bioavailability. In the present study, the minimum Phy:Fe value obtained was 13.7 which is much higher than the values reported by either Hurrell and Egli (2010) or Frontela et al., (2009) indicating that even if some of the analyzed landraces exhibit a relatively high Fe content, this mineral is poorly available for human absorption in durum wheat. Regarding the Phy:Zn molar ratio, the International Zinc Nutrition Consultative Group (2004) divided the obtained values into three groups: <5, 5-15, and >15, representing high, moderate and low absorption levels, respectively. According to this classification, 69% of the Iranian landraces and 77% of the Mexican landraces exhibited moderate Zn absorption (Supplementary Table 1) indicating that some of the landraces analyzed here could be effectively used by breeders to obtain varieties with improved Zn bioavailability. For this activity, the positive correlation found between phytate and ZnC will be an inconvenient. Strong selection would need to be applied to break the barrier of this association.

From the analysis of the phenolic acid contents obtained in the present study, in general both the Mexican and Iranian landraces exhibited lower concentrations of phenolic acids compared to previous studies. Specifically, Laddomada et al. (2017) reported an average total phenolic acid content of ~ 800 μg/g in a set of tetraploid wheat accessions whereas Shewry and Hey (2015) reported an average total phenolic acid concentration of 857 μg/g in 17 durum wheat lines and of 961 μg/g in a set of 44 emmer cultivars. Overall, these results suggest that the landraces analyzed here do not exhibit an exceptional quantity of phenolic acids and that wide variation of these compounds is typically identified across genotypes and environments indicating the complexity of this trait. As reported in previous studies (Li et al., 2008; Dinelli et al., 2009; Fernandez-Orozco et al., 2010; Laddomada et al., 2017) ferulic, sinapic and *p*-coumaric acids were, in order, the most abundant phenolic acids identified in wheat. When analyzing the correlations between each phenolic acid and the AX content, ferulic acid appeared to be positively associated with arabinoxylan content. Similar results were also reported by Marcotuli et al., (2016) and are probably associated with the disposition that exists to form covalent bonds between WE-AX and ferulic acid, which result in the formation of feruloylated arabinoxylans. The presence of ferulic acid linked to AX has been associated with antioxidant and prebiotic properties (Mendez-Encinas, Carvajal-Millan, Rascon-Chu, Astiazaran-Garcia, & Valencia-Rivera, 2018) and, for this reason, durum wheat lines with high levels of ferulic acid and arabinoxylans should be preferred in order to improve the nutritional quality of durum wheat related products (Marcotuli et al., 2016). Also, a strong positive correlation between either p-hydroxybenzoic, ferulic, *p*-coumaric, sinapic acids and TOT-AX content was found. Similar correlations were previously identified by Marcotuli et al., (2016) and are probably determined by the fact that cereal

arabinoxylans can include in their structure hydroxycinnamic acid substituents such as ferulic and coumaric acids.

5. Conclusions

In the present study a set of Mexican and Iranian durum wheat landraces was analyzed for arabinoxylan, iron, zinc, phytic acid and phenolic acids content variations. Wide variation was identified for all the traits and specific accessions showing desirable combination of values were found. The landraces identified in the present study could be a useful resource for breeders who aim to improve the wheat nutritional quality and especially the content of dietary fibers, micronutrients and their relative bioavailability.

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References:

Alvarez, J. B., & Guzmán, C. (2018). Interspecific and intergeneric hybridization as a source of variation for wheat grain quality improvement. *Theoretical and Applied Genetics*, *131*, 225–251. https://doi.org/10.1007/s00122-017-3042-x

American Association of Cereal Chemists. 2010. Approved methods of the AACC. St.Paul, MN, USA

Ciccoritti, R., Scalfati, G., Cammerata, A., & Sgrulletta, D. (2011). Variations in content and extractability of durum wheat (*Triticum turgidum* L. var *durum*) arabinoxylans associated with genetic and environmental factors. *International Journal of Molecular Sciences*, *12*, 4536–4549. https://doi.org/10.3390/ijms12074536

De Santis, M. A., Kosik, O., Passmore, D., Flagella, Z., Shewry, P. R., & Lovegrove, A. (2018). Comparison of the dietary fibre composition of old and modern durum wheat (*Triticum turgidum* spp. *durum*) genotypes. *Food Chemistry*, *244*, 304–310. https://doi.org/10.1016/j.foodchem.2017.09.143

Dinelli, G., Carretero, A. S., Di Silvestro, R., Marotti, I., Fu, S., Benedettelli, S., … Gutiérrez, A. F. (2009). Determination of phenolic compounds in modern and old varieties of durum wheat using liquid chromatography coupled with time-of-flight mass spectrometry. *Journal of Chromatography A*, *1216*, 7229–7240. https://doi.org/10.1016/j.chroma.2009.08.041

Douglas, S.G. (1980) A rapid method for the determination of pentosans in wheat flour. *Food Chemistry, 7*,139-145

Eagling, T., Wawer, A. A., Shewry, P. R., Zhao, F., & Fairweather-tait, S. J. (2014). Iron bioavailability in two commercial cultivars of wheat: comparison between wholegrain and white flour and the effects of nicotianamine and 2 ′ -deoxymugineic acid on iron uptake into Caco - 2 Cells. *Journal of Agricultural and Food Chemistry*, *62*, 10320–10325.

Fernandez-Orozco, R., Li, L., Harflett, C., Shewry, P. R., & Ward, J. L. (2010). Effects of environment and genotype on phenolic acids in wheat in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, *58*, 9341–9352. https://doi.org/10.1021/jf102017s

Ficco, D. B. M., Riefolo, C., Nicastro, G., De Simone, V., Di Gesù, A. M., Beleggia, R., … De Vita, P. (2009). Phytate and mineral elements concentration in a collection of Italian durum wheat cultivars. *Field Crops Research*, *111*, 235–242. https://doi.org/10.1016/j.fcr.2008.12.010

Finnie, S. M., Bettge, A. D., & Morris, C. F. (2006). Influence of cultivar and environment on water-soluble and water-insoluble arabinoxylans in soft wheat. *Cereal Chemistry*, *83*, 617–623. https://doi.org/10.1094/CC-83-0617

Frontela, C., Scarino, M. L., Ferruzza, S., Ros, G., & Martínez, C. (2009). Effect of dephytinization on bioavailability of iron, calcium and zinc from infant cereals assessed in the Caco-2 cell model. *World Journal of Gastroenterology*, *15*, 1977–1984. https://doi.org/10.3748/wjg.15.1977

Gebruers, K., Dornez, E., Boros, D., Fraś, A., Dynkowska, W., Bedo, Z., … Courtin, C. M. (2008). Variation in the content of dietary fiber and components thereof in wheats in

436 the HEALTHGRAIN Diversity Screen. *Journal of Agricultural and Food Chemistry*,
437 *56*, 9740–9749. https://doi.org/10.1021/jf800975w

438 Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. *American*
439 *Journal of Clinical Nutrition*, *91*, 1461–1467.
440 https://doi.org/10.3945/ajcn.2010.28674F

441 International Zinc Nutrition Consultative Group (IZiNCG), 2004. Assessment of the risk of
442 zinc deficiency in populations and options for its control. In: Hotz, C., Brown, K.H.
443 (Eds.), Food and Nutrition Bulletin, vol. 25-Suppl. 2, pp:S114-S117.

444 Jurowski, K., Szewczyk, B., Nowak, G., & Piekoszewski, W. (2014). Biological
445 consequences of zinc deficiency in the pathomechanisms of selected diseases. *Journal*
446 *of Biological Inorganic Chemistry*, *19*, 1069–1079. https://doi.org/10.1007/s00775-
447 014-1139-0

448 Laddomada, B., Durante, M., Mangini, G., D'Amico, L., Lenucci, M. S., Simeone, R., …
449 Blanco, A. (2017). Genetic variation for phenolic acids concentration and composition
450 in a tetraploid wheat (*Triticum turgidum* L.) collection. *Genetic Resources and Crop*
451 *Evolution*, *64*, 587–597. https://doi.org/10.1007/s10722-016-0386-z

452 Laddomada, B., Durante, M., Minervini, F., Garbetta, A., Cardinali, A., D'Antuono, I., …
453 Mita, G. (2015). Phytochemical composition and anti-inflammatory activity of extracts
454 from the whole-meal flour of Italian durum wheat cultivars. *International Journal of*
455 *Molecular Sciences*, *16*, 3512–3527. https://doi.org/10.3390/ijms16023512

456 Li, L., Shewry, P. R., & Ward, J. L. (2008). Phenolic acids in wheat varieties in the
457 healthgrain diversity screen. *Journal of Agricultural and Food Chemistry*, *56*, 9732–
458 9739. https://doi.org/10.1021/jf801069s

459 Magallanes-López, A. M., Hernandez-Espinosa, N., Velu, G., Posadas-Romano, G.,
460 Ordoñez-Villegas, V. M. G., Crossa, J., … Guzmán, C. (2017). Variability in iron,
461 zinc and phytic acid content in a worldwide collection of commercial durum wheat
462 cultivars and the effect of reduced irrigation on these traits. *Food Chemistry*, *237*,
463 499–505. https://doi.org/10.1016/j.foodchem.2017.05.110

464 Marcotuli, I., Hsieh, Y. S. Y., Lahnstein, J., Yap, K., Burton, R. A., Blanco, A., …
465 Gadaleta, A. (2016). Structural variation and content of arabinoxylans in endosperm
466 and bran of durum wheat (*Triticum turgidum* L.). *Journal of Agricultural and Food*
467 *Chemistry*, *64*, 2883–2892. https://doi.org/10.1021/acs.jafc.6b00103

468 Mendez-Encinas, M. A., Carvajal-Millan, E., Rascon-Chu, A., Astiazaran-Garcia, H. F., &
469 Valencia-Rivera, D. E. (2018). Ferulated arabinoxylans and their gels: functional
470 properties and potential application as antioxidant and anticancer agent. *Oxidative*
471 *Medicine and Cellular Longevity*, *2018*. https://doi.org/10.1155/2018/2314759

Martini, D., Taddei, F., Ciccoritti, R., Pasquini, M., Nicoletti, I., Corradini, D., & D'Egidio, M. G. (2015). Variation of total antioxidant activity and of phenolic acid, total phenolics and yellow coloured pigments in durum wheat (*Triticum turgidum* L. var. durum) as a function of genotype, crop year and growing area. *Journal of Cereal Science*, *65*, 175–185. https://doi.org/10.1016/j.jcs.2015.06.012

Moore, M. A., Park, C. B., & Tsuda, H. (1998). Soluble and insoluble fiber influences on cancer development. *Critical Reviews in Oncology/Hematology*, *27*, 229–242. https://doi.org/10.1016/S1040-8428(98)00006-7

Shewry, P. R., & Hey, S. (2015). Do "ancient" wheat species differ from modern bread wheat in their contents of bioactive components? *Journal of Cereal Science*, *65*, 236–243. https://doi.org/10.1016/j.jcs.2015.07.014

Pasqualone, A., Delvecchio, L. N., Mangini, G., Taranto, F., & Blanco, A. (2014). Variability of total soluble phenolic compounds and antioxidant activity in a collection of tetraploid wheat. *Agricultural and Food Science*, *23*, 307–316. https://doi.org/10.23986/afsci.47985

Peña-Bautista, R. J., Hernandez-Espinosa, N., Jones, J. M., Guzmán, C., & Braun, H. J. (2017). CIMMYT Series on Carbohydrates, Wheat, Grains, and Health: Wheat-Based Foods: Their Global and Regional Importance in the Food Supply, Nutrition, and Health ,. *Cereal Foods World*, *62*, 231–249. https://doi.org/10.1094/CFW-62-5-0231

Velu, G., Crespo Herrera, L., Guzman, C., Huerta, J., Payne, T., & Singh, R. P. (2019). Assessing genetic diversity to breed competitive biofortified wheat with enhanced grain Zn and Fe concentrations. *Frontiers in Plant Science*, *9*, 1–11. https://doi.org/10.3389/fpls.2018.01971

Velu, G., Guzman, C., Mondal, S., Autrique, J. E., Huerta, J., & Singh, R. P. (2016). Effect of drought and elevated temperature on grain zinc and iron concentrations in CIMMYT spring wheat. *Journal of Cereal Science*, *69*, 182–186. https://doi.org/10.1016/j.jcs.2016.03.006

Figure captions

Figure 1. Number of landraces in each range of concentration of arabinoxylans, micronutrients, phytate, and phenolic acids in Iranian and Mexican landrace sets. Dotted lines indicates the average value for each of the traits.