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# Arsenic, lead and cadmium distribution in the pearled fractions of different winter wheat cultivars (*Triticum aestivum* L.)

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**KEYWORDS**

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## ABSTRACT

The pearling of wheat has been proposed as a technology to select intermediate bran fractions that can be employed in the production of functional foods. The aim of the study was to determine the distribution of arsenic, lead and cadmium in the pearled fractions of different types of common wheats in order to identify potential safety threats which may arise from the use of intermediate pearled fractions.

Arsenic, lead and cadmium were all found to be mainly concentrated in the outer layers of the kernel, but their distribution varied on an element-specific basis. The concentration of arsenic gradually decreased moving towards the internal layers of the kernel. Whereas, the concentration of lead dropped suddenly after the removal of the first fraction corresponding to 5% of the kernel weight. Cadmium was found to be distributed more uniformly in the grain and, after the removal of 25% of the kernel weight, the residual pearled kernel on average retained 56% of the cadmium detected in the whole grain.

A careful selection of the raw material, which should meet specific requirements especially in terms of cadmium contamination, should be made in order to avoid any potential risk for the health of consumers.

## 1. INTRODUCTION

Heavy metals, whose maximum levels in foodstuffs have been set by Commission Regulation (EC) No 1881/2006 and subsequent amendments, are some of the most dangerous contaminants of food. Cereal products are among the main dietary sources of some types of heavy metals because of their high consumption as staple foods (Galal-Gorchev, 1993).

Arsenic (As) has been classified, by the International Agency for Research on Cancer (IARC, 2017), as "carcinogenic to humans" (Group 1). In general, with the exception of the youngest population, grain-based processed products (non-rice-based) are considered the main contributor to the dietary exposure to inorganic arsenic (EFSA, 2014). Cadmium (Cd), which is classified as "carcinogenic to humans" (Group 1), occurs naturally at low levels in the environment, and foodstuffs represent the main source of cadmium exposure for non-smokers (Wu et al., 2016). Food is also one of the major sources of exposure to lead (Pb) (EFSA, 2012), whose inorganic form has been classified by the IARC as "probably carcinogenic to humans" (Group 2A).

As the outer parts of the kernel are generally the richest in inorganic elements, milling is expected to reduce the content of heavy metals in the flour fractions employed for food production (Cheli et al., 2010; Cubadda et al., 2003; De Brier et al., 2015; Guttieri et al., 2015; Zhao et al., 2010). However, by-products, such as the bran fraction, which is rich in bioactive compounds, represent a valuable raw material for the production of functional foods. Therefore, novel wheat fractionation technologies have been developed in order to better exploit the nutritional potential of these fractions and to meet food safety requirements (Delcour et al., 2012). In this regard, the pearling process was proposed not only as a wheat processing pretreatment to improve the removal of toxic elements (Cheli et al., 2010), but also as a technology to select intermediate bran fractions, which, at the same time, allows for the removal of parts of the grain that could be detrimental for technological quality and safety (Delcour et al., 2012; Giordano et al, 2016, 2017; Sovrani et al., 2012). Very little information is available about the distribution of heavy metals, such as arsenic, cadmium and lead, in the wheat kernel and in its derived pearled fractions. The aim of the present study was to determine the distribution of arsenic, cadmium and lead in the pearled fractions of different genotypes of common wheat grown over two different growing seasons, in order to analyse their distribution in the kernel and to identify any potential safety threats which may arise from the use of an intermediate bran fraction for food production.

## 2. MATERIALS AND METHODS

### 2.1 Experimental design

Four commercial winter wheat cultivars with different technological traits were sown in 2013 in North West Italy (Cigliano, Piedmont; 45°18'33''N, 8°01'09''E) in a loam and shallow soil, according to a completely randomized block design with three replications. The plot size was 8 x 1.5 m (12 $m^2$), and conventional agronomic techniques were adopted for the field experiments. Briefly, the previous crop was maize, and the mechanical sowing was carried out on 2 November 2013, following an autumn ploughing (30 cm) and disk harrowing to prepare a proper seedbed. Before sowing, all the plots received the same amounts of nutrients: 15, 40 and 80 kg/ha of N, $P_2O_5$ and $K_2O$, respectively. Moreover, at the tillering stage, 140 kg N/ha was applied as a slow-release nitrogen fertiliser (Entec® 46, EuroChem Agro, Cesano Maderno, Italy). All the plots were sprayed at the flowering stage with prothioconazole [Proline®, Bayer, Italy, emulsifiable concentrate formulation (EC), which was applied at 0.200 kg of active ingredient (AI)/ha] to control fungal disease. The mechanical harvesting was carried out on 7 July 2014, by means of a Walter Wintersteiger cereal plot combine-harvester.

The compared wheat cultivars were:

- Bramante (Società Italiana Sementi, Bologna, Italy): a soft wheat variety classified, according to the Italian bread-making quality grade, as wheat for biscuits;
- PR22R58 (Pioneer Hi-Bred Italia S.r.l, Cremona, Italy): a medium hard wheat variety classified as bread making-quality wheat;

- Rebelde (Apsovsementi S.p.A, Voghera, Italy): a hard wheat variety classified as improver wheat, with a high protein content.
- Project W (Società Produttori Sementi, Bologna, Italy): a medium hard waxy wheat.

The same experimental setup was followed over the 2014-15 growing season, in a different field of the same location, but this time only Bramante and PR22R58 cvs. were compared because of their large diffusion on the Italian market. The sowing was carried out on 3 November 2014, whereas the harvesting was conducted on 29 June 2015. The other agronomical practices were the same as those of the previous growing season. During both the investigated periods, rainfall and temperature data were recorded daily in a weather station, located next to the experimental field.

## 2.2 Soil samplings and analyses

The top soil layer (0-30 cm) of the fields sown in the 2 growing seasons was collected by means of Eijkelkamp cylindrical augers at wheat sowing. In each growing season 20 samples were collected in different areas of the experimental field. After pooling together the samples, the main physical and chemical characteristics of the soil and the contents of arsenic, cadmium and lead were determined as reported in the Italian Regulation D.M. 13 September 1999.

## 2.3 Wheat grain characterization

Thousand kernel weight (TKW) was determined on three 100-kernel sets of each sample, using an electronic balance. Test weight (TW) was determined by means of a Dickey-John GAC2000 grain analysis meter (Dickey-John Corp., Auburn, IL), using the supplied program, after validation with reference materials.

## 2.4 Wheat pearling

Six pearled fractions of the kernels were obtained through an incremental pearling of the grains, according to the approach described by Sovrani et al. (2012). The pearling consisted of consecutive passages of kernels and pearled kernels in an abrasive-type grain testing mill (Model TM-05C, Satake, Tokyo, Japan). Starting from unprocessed wheat grain (5.5 kg, obtained by pooling an aliquot of grain from each plot), the kernels were initially pearled to remove 5% of the original grain weight, and this resulted in a first fraction (0-5% w/w). The remaining kernels were pearled to remove a second fraction of 5% (5-10% w/w). The pearling process was repeated to remove a third, fourth and fifth fraction (designed fractions of 10-15%, 15-20%, 20-25% w/w). A residual 75% of the kernel (25-100% w/w) was also collected. The pearling process was performed at the constant speed of 55 Hz, and the estimation of the time necessary to remove 5% of kernel weight at each pearling passage was experimentally quantified for each cultivar. The pearling process was then monitored by means of a time control, and after each pearling session, the laboratory pearler was cleaned thoroughly to minimize equipment contamination. All the unprocessed grain samples (0.5 kg) and the residual pearled kernel were milled using a laboratory centrifugal mill (Model ZM-100, Retsch, Haan, Germany) with a 1-mm opening. Prior to the chemical analyses, all the samples were ground to a fine powder (particle size < 500 μm) with a Cyclotec 1093 sample mill (Foss, Padova, Italy), and stored at -25°C until analyses were performed.

## 2.5 Analysis of the ash, arsenic, lead and cadmium contents of the wheat fractions

The ash content was determined in a muffle furnace according to the AOAC 923.03 procedure. The arsenic, cadmium and lead analyses were performed as described by UNI EN 15763:2010 “Foodstuffs – Determination of trace elements – Determination of arsenic, cadmium, mercury and lead in foodstuff by inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion”. The moisture of each sample was determined in order to express the results on a dry weight (dw) basis.

## 2.6 Statistical analyses

All the analyses of wheat samples (whole-grain flour, pearling fractions and residual pearled kernel) were performed in triplicate. Analysis of variance (ANOVA) was applied in order to compare the ash, arsenic, lead and cadmium contents in the whole-grain flours and in the different pearled fractions. The REGW-Q test was performed for multiple comparisons. A 0.05 threshold was used to reject the null hypothesis.

Statistical analyses were carried out by means of SPSS for Windows, statistical package Version 24 (SPSS Inc., Chicago, Illinois).

## 3. RESULTS

### 3.1 Meteorological conditions

The meteorological conditions observed in the experimental areas over the two growing seasons are reported in Table 1. The cumulative rainfall was different over the two growing seasons in the period between wheat sowing (November) and harvesting (June). The 2013-14 growing season was characterized by a lower level of cumulative rainfall than the 2014-15 season. Moreover, the first growing season showed a more homogenous rainfall distribution over the crop cycle, while almost half of the rainfall in the 2014-15 growing season was concentrated in the first month after sowing. The GDDs of the two growing seasons were similar, but the average daily temperature was generally higher in the second growing season.

### 3.2 Physical and chemical properties of the top soil layers

The physical and chemical properties of the top soil layers (0-30 cm) of the experimental fields sown in the two growing seasons are reported in Table 2.

The top soil layer of the field sown in the 2014-15 growing season showed a higher organic matter and total nitrogen than the field sown in the 2013-14 growing season. The soil pH was close to neutral and sub-acidic for the two seasons, respectively. The heavy metal contamination was found to be higher in the soil of 2013-14 growing season, as far as the concentrations of arsenic (+13%) and lead (+77%) are concerned. The cadmium contamination of the soils was similar for the two growing seasons.

### 3.3 Physical characteristics of the grains and the ash and heavy metal contents of their whole-grain flours

The physical characteristics of the grains as well as the ash and heavy metal concentrations of their whole-grain flours are reported in Table 3.

Significant differences were observed for the main grain yield and quality parameters of the wheat cultivars compared over the two growing seasons. A high grain yield was observed in the 2013-14 growing season for all the cultivars (> 8 t/ha). In particular, the Bramante and PR22R58 cvs. showed grain yields higher than 9 t/ha in the first growing season, while a lower grain yield was observed in the 2014-15 growing season. The low yield of the second growing season was ascribed to the high rainfall level occurred during the first vegetative growth stages which caused a final low plant density (data not showed), and the high temperatures associated to low rainfall levels observed at the end of the growing cycle which caused a low TKW.

The ash content observed in the whole-grain flour was on average 15.3 mg/g dw, and differences were observed among the analysed cultivars. As far as the heavy metal concentrations are concerned, cadmium was detected at the highest concentration (mean value: 0.101 mg/kg dw). The lowest cadmium contamination was observed in the Rebelde and Project W cvs. (0.082 and 0.083 mg/kg dw), while the Bramante and PR22R58 cvs. showed the highest content (>0.100 mg/kg dw). Lead was detected at a concentration 8 times lower than cadmium (mean value: 0.013 mg/kg dw), and the highest concentration was find in the Bramante and Rebelde cvs. harvested in 2014. Arsenic was not detected in any of the whole-grain flours. As far as the comparison between the growing seasons is concerned (cvs. Bramante and PR22R58), the concentration of lead and cadmium was on average 40% higher and 8% lower in the first growing season than in the second one.

## 3.4 Arsenic, cadmium and lead contents in the wheat pearled fractions

The influence of the three main factors (genotype, pearled fraction and growing season) on the ash, arsenic, lead and cadmium contents of the pearled fractions was evaluated by means of a three-way ANOVA (Table 4). The pearled fraction accounted for more than 80% of the variation observed for both arsenic, lead and cadmium content (Figure 1). The growing season influenced significantly only the lead and cadmium content accounting for 8.8% and 4.1% of the variation, respectively. Contrarily, the genotype showed a significant influence only on the cadmium content of pearled fractions, accounting for 3.0% of the variation.

The ash content was found to be the highest in the outer 20% of the grain, while the lowest concentration was observed in the residual pearled kernel (Table 5). Arsenic was only detected in the outermost 0-5% and 5-10% fractions with the exception of the Bramante and PR22R58 cvs. harvested in 2014 and 2015, respectively. In particular, arsenic was detected up to the third 10-15% pearled fraction in the former cultivar, whereas it was detected up to the fourth 15-20% fraction in the latter one. Regardless of which cultivar was considered, the arsenic concentration significantly decreased from the outermost to the innermost fractions, and it was never detected in the residual pearled kernel.

All the cultivars showed the highest concentration of lead in the 0-5% pearled fraction, whereas it was not detected in the residual 25-100% pearled kernel. In particular, the lead concentration in the 0-5% fraction was from 8 to 23-fold higher than the concentration detected in the whole grain. The Bramante cv. harvested in 2014 showed a significant gradual decrease in the lead concentration from the outermost pearled fraction to the 10-15% fraction; even though a gradual decrease in lead concentration was also observed in the 15-20% and 20-25% fractions, these two fractions did not differ significantly from the 10-15% one. The same cultivar collected in 2015 showed a lead content in the first 0-5% fraction two times lower than the one observed in the first growing season. After a significant decrease of lead concentration at each pearling step, lead was no longer detected from the 15-20% fraction onwards. Similarly, the lead concentration of PR22R58 cv. decreased significantly until the 15-20% fraction in the 2013-14 growing season, even though a further decrease was observed moving towards the 20-25% fraction. The same cultivar collected in the 2014-15 growing season showed a two-fold lower lead content in the 0-5% pearled fraction, and a gradual decrease until the 20-25% fraction.

Unlike arsenic and lead, cadmium was detected in all the pearled fractions of all the cultivars. Cadmium was found to be mainly concentrated in the peripheral layers of the kernel, with a decrease in its concentration after each pearling step. The only exception was the Bramante cv. harvested in 2015, which showed the highest cadmium concentration in the 5-10% pearled fraction instead of in the 0-5% one. Even though the lowest cadmium concentration in all the analyzed wheat samples was observed in the residual pearled kernel, this fraction retained from 56% to 60% of the cadmium detected in the whole grain. The only exception was the PR22R58 cv. harvested in 2014, whose residual pearled kernel retained 48% of the cadmium detected in the whole-grain flour.

## 4. DISCUSSION

The relevance of heavy metals on human health prompted the European Community to establish their maximum levels in foodstuffs. Commission Regulation (EC) No 1881/2006 and 488/2014 established a specific limit in wheat for both cadmium (0.20 mg/kg fresh weight for wheat grain, germ and bran) and for lead (0.20 mg/kg fresh weight for cereals). Even though EFSA (2014) reported that the main contributor to the dietary exposure to inorganic arsenic in Europe was the “Grain-based processed products (non-rice-based)” food group, because of its relatively high consumption levels, there are currently no maximum arsenic content levels in wheat grains and their derived products at an EU level. In the present study, the whole-grain flours of different wheat cultivars, collected in the 2013-14 and 2014-15 growing seasons, were found to have cadmium and lead concentrations that were under the established levels, whereas arsenic was not detected in any of the whole-grain samples. The average lead and cadmium content observed in the whole-grain flours was 0.013 and 0.101 mg/kg dw, respectively. The contamination levels observed were in accordance with the previously reported ones for common wheat grains grown in the same district in North Italy (Conti et al., 2000). The cadmium content was found to be about 33% higher than the one previously reported for the same industrialized area, whereas the lead concentration was almost the same. The analysis of different wheat genotypes, which clearly differed according to their productive and qualitative traits, showed that significant differences could occur in the lead and cadmium contents of their whole-grain flour. These differences can be mainly attributed to the different routes of entry of these metals in the wheat grain. The transfer of lead from soil to crop tissues is generally low because of soil retains most of the lead (McLaughlin et al., 1999), and consequently most of the lead comes from atmospheric deposition (Dalenberg and Vandriel, 1990; Zhao et al., 2004). According to the distribution of lead within the kernel layers, in the first growing season the smaller was the kernel size, expressed as TKW, the higher was the lead contamination of the whole-grain flour because of a higher surface/volume ratio. Nevertheless, in the second growing season, characterized by low lead contamination levels, the same relationship was not observed in the two cultivars tested.

Contrarily to lead, even if the cadmium of a wheat kernel may come from atmospheric fall-out, it could be derived also from soil absorption and transfer to the kernel, with a concentration factor from the soil usually in the range of 0.1 to 1-fold (Lübben and Sauerbeck, 1991; Mench, et al., 1997). In accordance with previous studies, the concentration factor from the soil to the kernel detected in the present study varied between 0.2 to 0.3-fold. Moreover, whole-grain samples collected in the second growing season showed on average a higher cadmium concentration than samples collected in the first growing season (0.115 mg/kg vs. 0.094 mg/kg dw). In fact, it could be supposed that, even if the content of cadmium of soil in the two growing seasons was very similar, the higher content of exchangeable calcium, magnesium and potassium observed in the first growing season may have negatively affected cadmium uptake by plants (McLaughlin et al., 1999). On the contrary, a genetic variability in cadmium accumulation in wheat grain (Kubo et al., 2008, 2011) could be responsible of the differences observed between different cultivars within the same growing season.

As far as the distribution pattern of arsenic, lead and cadmium in the pearled fractions is concerned, results clearly shows that the concentration of these elements was mainly related to the milling rate, and consequently to how unevenly the specific element was distributed in the kernel (Figure 1 and 2). Although the arsenic content was under the quantification limit in the whole-grain flour, it was detected in the outer layers of the kernel of all the cultivars, with a gradual reduction moving towards the endosperm. This element was no longer detected from the 10-15% fraction onwards with the exception of the Bramante and PR22R58 cvs. collected in 2014 and 2015, respectively. The obtained results were in agreement with those of Zhao et al. (2010), who concluded that although the bran fraction in wheat varieties, characterized by a higher arsenic content than the ones of the present study, accounted for only 23-29% of the total grain weight, it contained 53-65% of the total arsenic of the grain.

Lead was found to be mainly concentrated in the 0-5% pearled fraction, which retained on average 66% of the content observed in the whole grain. Lead concentration drastically decreased after the removal of this first external fraction, and the retained content in the 5-10%, 10-15%, 15-20% and 20-25% fractions was 17%, 11%, 10% and 8%, respectively.

In agreement with previous studies performed on wheat flour after conventional roller-milling (Cheli et al., 2010; Guttieri et al., 2015), and contrarily to arsenic and lead, cadmium was found to be more uniformly distributed within the wheat grain, and the residual pearled kernel retained about 56% of the cadmium detected in the whole grain.

## 5. CONCLUSIONS

The results of the present study clearly show that the distribution of heavy metals in wheat grain pearled fractions varies on an element-specific basis. Both arsenic and lead are unevenly distributed in the kernel, and their concentrations could be greatly reduced after the removal of 10% or 5% of the kernel weight, respectively. On the contrary, cadmium is more uniformly distributed in the grain, and its concentration is affected less by the pearling process.

These results should be taken into account in the production of functional ingredients from intermediate bran layers by means of a pearling process. Ten percent of the grain mass should be discarded because of the high concentration of lead and arsenic in this fraction. This procedure could allow about 83% of lead to be removed. At the same time, the removal of the same fraction could also be useful to reduce the concentration of other contaminants, such as mycotoxins and microorganisms, which are characterized by a similar distribution pattern (Bottega et al., 2009; Laca et al., 2006; Sovrani et al., 2012). Nevertheless, only 18% of cadmium could be removed after discharging the 0-10% pearled fraction. Thus, great attention should be paid to the use of an intermediate bran fraction, because it may pose a risk for consumer safety, particularly in terms of cadmium contamination. Although, the cadmium concentration of the pearled fractions analyzed was on average lower than the established European maximum levels, this threshold might be overcome in the outer pearled fractions obtained from wheat grains grown in soils characterized by higher levels of cadmium (Wang et al., 2011). In conclusion, the use of the pearling process to select bran fractions as a source of healthy ingredients needs to be associated with an accurate selection of the raw material, which has to meet specific requirements, especially in terms of cadmium contamination, in order to avoid any potential risks for consumers' health. Further study will be necessary in order to transfer the results to an industrial scale and optimize the pearling process for the production of functional ingredients by removing the parts of the grain detrimental in terms of heavy metal contamination.

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## 9. TABLES AND FIGURES

**Table 1.** Cumulative rainfall, rainy days, ADT (Average Daily Temperature) and GDDs (Growing Degree Days) measured in the experimental area from sowing (November) to harvesting (June) in the 2013-2015 period.

| Growing season | Month | Cumulative rainfall (mm) | Rainy days | ADT (°C) | GDDs[a] |
|---|---|---|---|---|---|
| 2013-14 | November | 68 | 17 | 7 | 253 |
| | December | 139 | 14 | 3 | 169 |
| | January | 117 | 17 | 4 | 149 |
| | February | 129 | 15 | 6 | 179 |
| | March | 71 | 8 | 10 | 335 |
| | April | 138 | 9 | 14 | 428 |
| | May | 84 | 15 | 16 | 515 |
| | June | 144 | 18 | 21 | 637 |
| | November-June | 890 | 113 | 10 | 2665 |
| 2014-15 | November | 438 | 24 | 10 | 303 |
| | December | 89 | 23 | 5 | 184 |
| | January | 36 | 7 | 4 | 133 |
| | February | 75 | 10 | 4 | 143 |
| | March | 88 | 9 | 9 | 304 |
| | April | 75 | 10 | 14 | 409 |
| | May | 96 | 12 | 18 | 569 |
| | June | 86 | 11 | 22 | 659 |
| | November-June | 983 | 106 | 11 | 2704 |

[a] Accumulated growing degree days for each month using a 0°C base.

**Table 2.** Physical and chemical properties of the top soil layers (0-30 cm) in the experimental areas (2013-14 and 2014-15 growing seasons).

| | | Growing season | |
|---|---|---|---|
| Parameter | Unit | 2013-14 | 2014-15 |
| Sand (2000 – 50 µm) | % | 37.2 | 44.2 |
| Silt (50 – 2 µm) | % | 53.8 | 46.4 |
| Clay (< 2 µm) | % | 9.0 | 9.4 |
| Cation-exchange capacity | meq/100g | 11.4 | 10.6 |
| Total limestone | % | 0.8 | 0.8 |
| Active limestone | % | 0.3 | 0.4 |
| pH | | 6.7 | 5.8 |
| Organic matter | % | 1.55 | 1.93 |
| Total nitrogen | % | 0.084 | 0.098 |
| Assimilable phosphorus | mg/kg | 23 | 89 |
| Exchangeable potassium | mg/kg | 164 | 114 |
| Exchangeable calcium | mg/kg | 1277 | 595 |
| Exchangeable magnesium | mg/kg | 118 | 33 |
| Arsenic | mg/kg | 7.75 | 6.85 |
| Lead | mg/kg | 30.70 | 17.30 |
| Cadmium | mg/kg | 0.38 | 0.39 |

**Table 3.** Yield, TKW (Thousand Kernel Weight) and TW (Test Weight) of grains collected in the two growing seasons, and the ash, arsenic, lead and cadmium contents of the respective whole-grain flours.

| | | | Yield | TKW | TW | Ash | Arsenic | Lead | Cadmium |
|---|---|---|---|---|---|---|---|---|---|
| Cultivar name | Growing season | Qualitative classification | (t/ha) | (g) | (kg/hL) | (mg/g) | | (mg/kg) | |
| Bramante | 2013-14 | Wheat for biscuits | 9.0 a | 43.1 b | 78.2 bc | 15.2 ab | n.d.[a] | 0.019 a | 0.103 c |
| PR22R58 | 2013-14 | Bread-making quality wheat | 9.2 a | 51.7 a | 75.1 d | 16.4 a | n.d. | 0.009 c | 0.108 bc |
| Rebelde | 2013-14 | Improver wheat | 8.3 a | 39.2 b | 79.3 ab | 17.6 a | n.d. | 0.021 a | 0.082 d |
| Project W | 2013-14 | Waxy wheat | 8.0 a | 49.2 a | 77.9 c | 13.2 b | n.d. | 0.008 c | 0.083 d |
| Bramante | 2014-15 | Wheat for biscuits | 4.5 b | 35.1 c | 79.9 a | 12.8 b | n.d. | 0.008 c | 0.116 a |
| PR22R58 | 2014-15 | Bread-making quality wheat | 5.7 b | 42.7 b | 78.0 bc | 16.6 a | n.d. | 0.012 b | 0.113 ab |
| SEM[b] | | | 1.5 | 3.5 | 1.2 | 2.0 | - | 0.002 | 0.005 |
| P (F) | | | <0.001 | <0.001 | <0.001 | <0.001 | - | <0.001 | <0.001 |

Means (n=3) within a column followed by different letters are significantly different, according to the REGW-Q test (the ANOVA level of significance is shown in the table). The results of the ash as well as the arsenic, lead and cadmium contents are expressed on a dw basis.

[a] n.d.: not detected

[b] SEM: standard error of the mean

**Table 4.** Levels of significance of the three-way ANOVA analyses performed using data of Bramante and PR22R58 cvs. collected in the 2013-14 and 2014-15 growing seasons.

| Source of variation | Ash | Arsenic | Lead | Cadmium |
|---|---|---|---|---|
| Genotype | **<0.001** | 0.436 | 0.472 | **<0.001** |
| Pearled fraction | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| Growing season | **0.036** | 0.228 | **<0.001** | **<0.001** |

Significant values are reported in bold style.

**Table 5.** Ash, arsenic, cadmium and lead contents in the wheat pearled fractions.

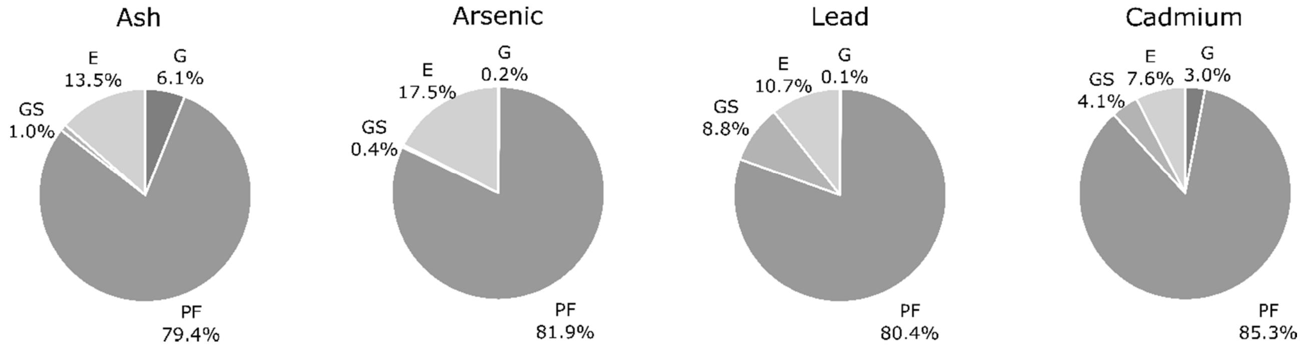
| Cultivar name | Growing season | Pearled fraction | Ash (mg/g) | Arsenic (mg/kg) | Lead (mg/kg) | Cadmium (mg/kg) |
|---|---|---|---|---|---|---|
| Bramante | 2013-14 | 0-5% | 28.3 c | 0.022 a | 0.250 a | 0.202 a |
| | | 5-10% | 34.5 a | 0.015 b | 0.066 b | 0.174 b |
| | | 10-15% | 31.8 b | 0.007 c | 0.038 c | 0.161 c |
| | | 15-20% | 24.3 d | n.d.[a] | 0.037 c | 0.134 d |
| | | 20-25% | 18.0 e | n.d. | 0.033 c | 0.119 e |
| | | 25-100% | 15.0 f | n.d. | n.d. | 0.082 f |
| | | SEM[b] | 1.4 | 0.002 | 0.016 | 0.009 |
| | | P (F) | <0.001 | <0.001 | <0.001 | <0.001 |
| PR22R58 | 2013-14 | 0-5% | 31.7 a | 0.015 a | 0.180 a | 0.150 a |
| | | 5-10% | 30.2 ab | 0.011 b | 0.064 b | 0.147 a |
| | | 10-15% | 31.2 a | n.d. | 0.042 c | 0.129 b |
| | | 15-20% | 28.3 ab | n.d. | 0.028 d | 0.128 b |
| | | 20-25% | 25.6 b | n.d. | 0.021 d | 0.110 c |
| | | 25-100% | 10.1 c | n.d. | n.d. | 0.069 d |
| | | SEM | 4.1 | 0.001 | 0.005 | 0.006 |
| | | P (F) | <0.001 | 0.001 | <0.001 | <0.001 |
| Rebelde | 2013-14 | 0-5% | 46.0 a | 0.014 a | 0.181 a | 0.199 a |
| | | 5-10% | 35.2 b | 0.010 b | 0.048 b | 0.179 b |
| | | 10-15% | 33.7 b | n.d. | 0.034 c | 0.147 c |
| | | 15-20% | 27.7 c | n.d. | 0.019 d | 0.130 d |
| | | 20-25% | 22.6 d | n.d. | 0.016 d | 0.110 e |
| | | 25-100% | 5.5 e | n.d. | n.d. | 0.065 f |
| | | SEM | 3.5 | 0.001 | 0.003 | 0.004 |
| | | P (F) | <0.001 | 0.008 | <0.001 | <0.001 |
| Project W | 2013-14 | 0-5% | 41.9 b | 0.016 a | 0.185 a | 0.177 a |
| | | 5-10% | 45.7 a | 0.008 b | 0.035 b | 0.170 b |
| | | 10-15% | 36.7 c | n.d. | 0.014 d | 0.141 c |
| | | 15-20% | 26.8 d | n.d. | 0.022 c | 0.134 d |
| | | 20-25% | 26.0 d | n.d. | 0.012 d | 0.102 e |
| | | 25-100% | 7.1 e | n.d. | n.d. | 0.064 f |
| | | SEM | 3.0 | 0.001 | 0.004 | 0.005 |
| | | P (F) | <0.001 | <0.001 | <0.001 | <0.001 |
| Bramante | 2014-15 | 0-5% | 33.5 a | 0.012 a | 0.125 a | 0.206 b |
| | | 5-10% | 30.9 ab | 0.006 b | 0.021 b | 0.214 a |
| | | 10-15% | 29.5 b | n.d. | 0.010 c | 0.155 c |
| | | 15-20% | 22.1 c | n.d. | n.d. | 0.133 d |
| | | 20-25% | 15.7 d | n.d. | n.d. | 0.116 e |
| | | 25-100% | 9.1 e | n.d. | n.d. | 0.089 f |
| | | SEM | 3.1 | 0.001 | 0.002 | 0.005 |
| | | P (F) | <0.001 | 0.003 | <0.001 | <0.001 |
| PR22R58 | 2014-15 | 0-5% | 39.2 b | 0.015 a | 0.100 a | 0.193 a |
| | | 5-10% | 45.5 a | 0.010 b | 0.030 b | 0.185 b |
| | | 10-15% | 37.3 c | 0.009 b | 0.035 b | 0.165 c |
| | | 15-20% | 32.0 d | 0.007 c | 0.023 c | 0.141 d |
| | | 20-25% | 24.6 e | n.d. | 0.012 d | 0.119 e |
| | | 25-100% | 11.8 f | n.d. | n.d. | 0.084 f |
| | | SEM | 1.4 | 0.002 | 0.004 | 0.005 |
| | | P (F) | <0.001 | <0.001 | <0.001 | <0.001 |

Means (n=3) within a column followed by different letters are significantly different, according to the REGW-Q test (the ANOVA level of significance is shown in the table). The results of the ash as well as the arsenic, lead and cadmium contents are expressed on a dw basis.

[a] n.d.: not detected

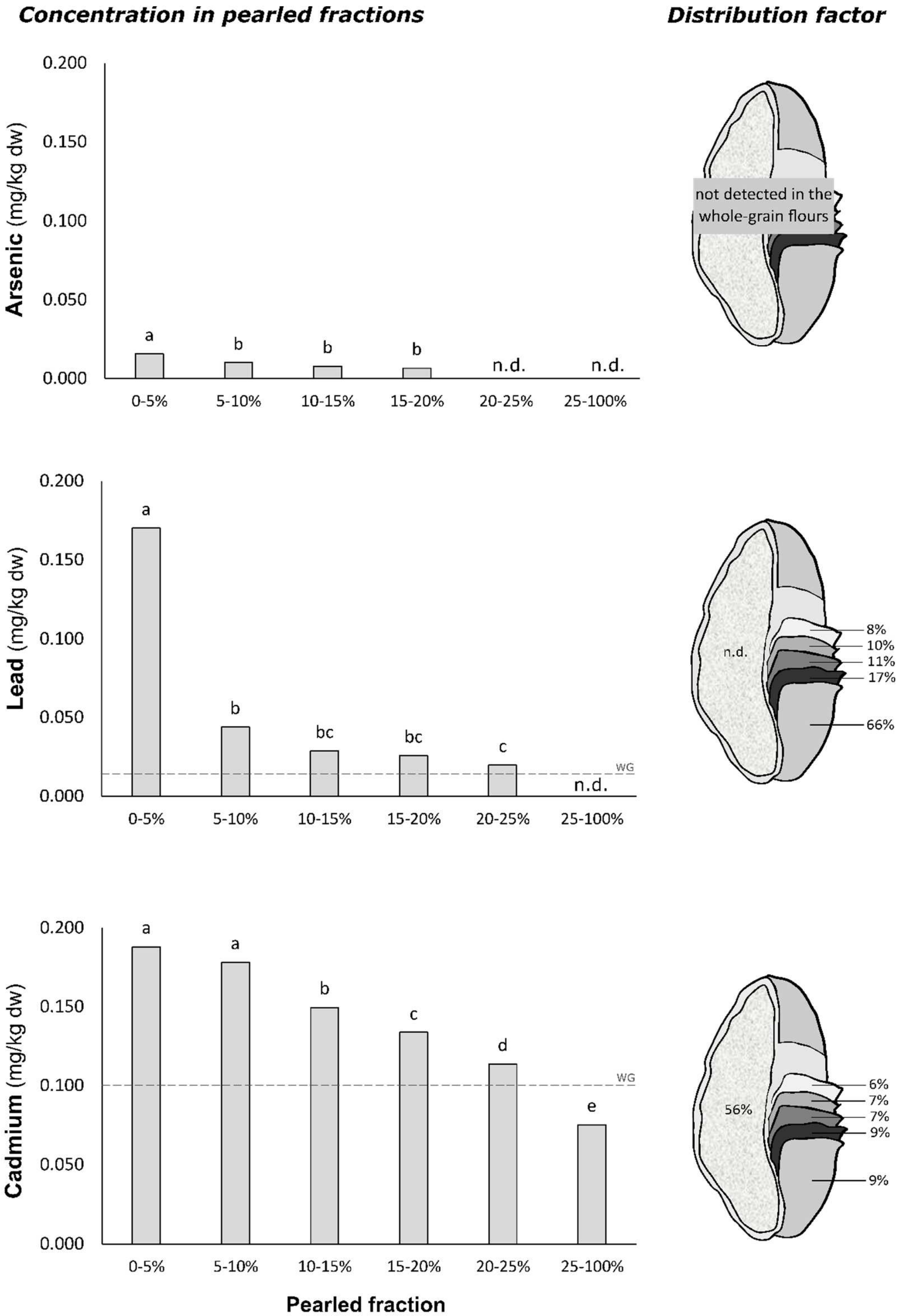
[b]SEM: standard error of the mean

**Figure 1.** Variance components on the content of ash, arsenic, lead and cadmium.




Variance (Var) components were calculated as the ratio $Var_{G,PF,GS,E}/(Var_G+Var_{PF}+Var_{GS}+Var_E)$, with $Var_G$, $Var_{PF}$, $Var_{GS}$ and $Var_E$ being the genotype (G), pearling fraction (PF), growing season (GS) and error (E) variances, respectively.

**Figure 2.** Average concentration and distribution factor of arsenic, lead and cadmium in the pearled wheat fractions.




**Left side** - The reported data are the mean values of the six wheat cultivars harvested in 2014 and 2015 (n=18), expressed on a dw basis. Values with different letters differ significantly (P (F) <0.001). The dashed line corresponds to average values observed in the whole-grain flour (WG); no dashed line is reported for arsenic because its concentration was under the limit of quantification in the whole-grain flour. **Right side** – The distribution factor was calculated considering both the heavy metal concentration and the relative weight of each fraction in comparison to the whole-grain flour.