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Inductively coupled plasma mass spectrometry with on-line leaching to assess the maximum bio-accessibility of toxic and essential elements in wheat from Saudi Arabia†

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For the first time, a continuous leaching method, coupled on-line with inductively coupled plasma mass spectrometry (ICPMS), was used on wheat samples to assess the maximum bio-accessibility of several trace elements. This technique involves the sequential continuous leaching of a mini-column of sample by artificial saliva, gastric juice and intestinal juice, while the effluent from the mini-column flows to the nebulizer of an ICPMS instrument. A larger mini-column than previously used with this method was utilized to improve reproducibility. The results of the continuous on-line leaching method were comparable to those of a conventional batch method despite the fact that it requires 15 min as opposed to several hours by the batch method. They show that bio-accessibility varies from element to element between samples. The bio-accessible fraction of the majority of elements (*e.g.* As, Fe, Ni, Cu, *etc.*) was mostly released by saliva and gastric juice. Particular attention was paid to Cd and Pb in wheat, because even a portion of these elements being bio-accessible could have major implications for consumers. The highest bio-accessible concentrations of Pb were found to be 780 ± 90 μg kg^{-1} and 890 ± 100 μg kg^{-1} for Safeer wheat and Qassim wheat, respectively, corresponding to 60% and 100% bio-accessibilities. Furthermore, the maximum Cd bio-accessible concentration was 1810 ± 280 μg kg^{-1} for Qassim wheat samples, corresponding to 100% bio-accessibility, which is a concern for consumption by children.

Introduction

Heavy metals (HMs), which are ubiquitous in the environment, may contaminate agricultural soils and crops (*e.g.*, wheat, rice, and fruits), thereby constituting an environmental problem because of their toxicity and bio-accumulation.[1] Vehicular exhaust, waste disposal, atmospheric deposition, fertilization, and use of pesticides increase the concentrations of HMs.[2,3] In Saudi Arabia, their level has also risen as a result of factory emissions, industrial and domestic waste waters as well as an increased number of automobiles.[4] Youssef and El-Sorogy analysed 45 sediment samples in Al-Kharrar lagoon in central western Saudi Arabia using inductively coupled plasma mass spectrometry (ICPMS) and found that the concentrations of Pb and Cd clearly exceeded the environmental background values.[4] High concentrations of Fe, Pb and Cu were also measured along the Red Sea coast in Jeddah, which may be attributed to pollution from oil refineries as well as sewage and fish wastes from local industries.[5] Furthermore, accumulation of HMs has been reported in fish.[6] Consequently, the level of HMs must be monitored in food to ensure its safety for consumption. Because the consumption of contaminated food may lead to the chronic accumulation of HMs in consumers' kidneys and liver, leading to serious diseases, regulations have been set for many food items.[7]

In Saudi Arabia, wheat is one of the main crops and is widely consumed by Saudis. Its popularity stems from its versatility, as it is used in pizza dough, different kinds of bread including hamburger buns, and traditional bakery products.[8] According to the United States Department of Agriculture (USDA), wheat flour is rich in several essential nutrients (*e.g.* Fe, Mn, Zn and Cu), which should be at suitable levels for enzymes to function properly in metabolic processes. An excessive amount of essential nutrients such as Mn may induce neurotoxic effects.[9] The average consumption of wheat being approximately 300 grams per day,[1,8] it may be an important source of essential and potentially toxic elements in the human diet.[1] The concentrations of HMs in wheat can reveal the ability of various wheat species to take HMs up and accumulate them.[1]

In Pakistan, the concentrations of Cr, Cu, Fe, Cd and Pb were reported to be high in wheat grains, although not at levels that could lead to cancer.[1] In China, the concentrations of HMs were lower than the Chinese legislation thresholds is some wheat

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samples, but were higher in others.[10] In central Argentina, the mean wheat grain concentrations of Cr, Cu, Fe, and Zn exceeded the permissible limits of agricultural standards, as a result of atmospheric deposition in the cases of Cr, Cu, Ni, Pb and Zn.[11] In Turkey, Yilmaz and Soylak analysed 38 traditional bread samples, but the concentration of several elements (Co, Cd, Ni and Pb) were below the detection limit of flame atomic absorption spectrophotometry.[12]

Although the total concentration of some elements may be high, their bio-availability (the fraction absorbed into the systemic circulation system, where toxic effects may ensue) should be taken into account. Given the ethical constraints associated with bio-availability methods and how complex and time-consuming they are, a simpler test would be useful. However, bio-availability is only equal to bio-accessibility (fraction dissolved in the gastro-intestinal tract) in the worst case scenario. Hence, bio-accessibility, which can be measured *in vitro* through sequential exposure to artificial gastro-intestinal reagents simulating the mouth, stomach and intestine may be measured for realistic risk assessment. With this approach, a large number of samples can be analysed without ethical constraint.[13–15]

However, only in a few studies has the bio-accessibility of elements in wheat been determined. The bio-accessibility of some elements, such as Zn and Fe, in wheatgrass, which is consumed as a dietary supplement, was measured by *in vitro* gastro-intestinal digestion procedures.[16] The study revealed that the bio-accessibility of elements was greatest from fresh wheatgrass and the lowest from wheat seeds. Lei *et al.* reported that the bio-availability of iron in two wheat flour samples from China was 37–54%.[17]

Conventionally, a batch method is used to determine bio-accessibility. Different experimental designs exist, which give different bio-accessibility data depending on temperature, pH, the chemical composition of digestive fluid, residence time, and solid/liquid ratio.[18] They usually do not use saliva for leaching even if saliva has significant leaching capability in many samples.[13–15,18–21]

An alternative to batch methods is a simple continuous on-line leaching method that involves sequentially pumping artificial saliva, gastric juice and intestinal juice through a mini-column of food while continuously monitoring the released elements by ICPMS. It was used to measure the bio-accessible concentrations of Zn and Pb in corn bran,[20] As in rice,[14,15] as well as As, Cr, Cd and Pb in whole wheat bread.[13] Because the sample is continuously exposed to fresh reagent, the dissolution equilibrium is shifted to the right, which speeds up the leaching process compared to that with batch methods, thereby providing similar results in 15 min instead of over 4 h.[13,14] Furthermore, contamination during sample manipulation is reduced because the whole process is carried out in a closed system. Moreover, on-line leaching provides real-time data on the leaching kinetics of elements. It thus offers several advantages over batch methods for risk assessment purposes.

In this study, for the first time, the bio-accessible fraction of both potentially toxic and essential elements was measured in wheat grains grown in different parts of Saudi Arabia. Their bio-accessibility was determined using the continuous on-line leaching method coupled to a quadrupole-based ICPMS detector. A larger mini-column of food was used than in previous on-line leaching studies of other food[13–15,20] to improve reproducibility and decrease back-pressure problems. The results were compared with those obtained using a conventional batch method, as the ultimate goal is to obtain similar results as with the batch method but in a much shorter time so that the on-line method may be widely used for risk assessment. Comparison to food safety regulations was finally done to assess the risk to human health.

Experimental

Reagents

All high-density polyethylene bottles and glassware were cleaned by soaking in 10% v/v nitric acid for 48 h, rinsing with doubly deionized water (DDW) and drying out at room temperature. All DDW came from an Arium Pro UV|DI water purification system (Sartorius Stedim Biotech, Göttingen, Germany). Samples and residues were digested with sub-boiled HNO_3 (ACS grade; Fisher Scientific, Ottawa, ON, Canada) and H_2O_2 (J. T. Baker, Phillipsburg, NJ, USA). All HNO_3 and HCl were purified with a DST-1000 sub-boiling distillation system (Savillex, Minnetonka, MN, USA). Multi-element standard solutions were prepared in each leaching matrix using 1000 mg L^{-1} mono-element solutions (SCP Science, Baie d'Urfé, QC, Canada). National Institute of Standards and Technology (NIST) SRM 8437 hard red spring wheat flour (Gaithersburg, MD, USA) was used to validate the method.

Artificial gastro-intestinal fluids were prepared as described previously.[13] For saliva, 6.8 g of KH_2PO_4 (ACS grade; Fisher Scientific) and 77 mL of 0.2 mol L^{-1} NaOH (ACS grade; BioShop, Burlington, ON, Canada) were mixed and diluted to 1 L using DDW after adjusting the pH to 6.5 using 0.2 mol L^{-1} NaOH. For gastric juice, 2.0 g of NaCl (ACS grade; BioShop), 3.2 g of pepsin (Sigma-Aldrich, Oakville, ON, Canada) and 7.0 mL of sub-boiled HCl (ACS grade; Fisher Scientific) were mixed and diluted to 1 L ($pH = 1.2$) using DDW. For intestinal fluid, 6.8 g of KH_2PO_4, 10 g of pancreatin (*i.e.*, a mixture of amylase, lipase and protease) (Sigma-Aldrich, St. Louis, MO, USA) and 77 mL of 0.2 mol L^{-1} NaOH were mixed and diluted to 1 L using DDW after adjusting the pH to 6.8 with 0.2 M NaOH.

Sample collection and preparation

Six different wheat samples (Table 1), representing popular brands grown in different areas (Fig. 1), were purchased from

Table 1 Names and origins of wheat samples

Sample no.	Common name	Region of Saudi Arabia
1	Dubai	North
2	Nakleeh	West
3	Najran	South-west
4	Noqrah	Center
5	Qassim	Center
6	Safeer	Center

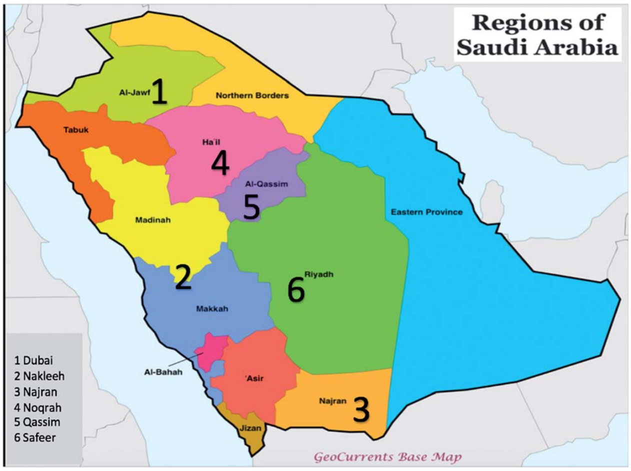

Fig. 1 Origin of wheat samples (picture taken from Google).

grocery stores in the Western province of Saudi Arabia. About 10 g of each sample was ground to a fine powder using porcelain mortar and pestle and stored in plastic bags (Ziplock) at 4 °C until use.

Instrumentation

A Varian 820MS ICPMS instrument (Varian Inc., Melbourne, Australia), equipped with a Scott double-pass spray chamber, Burgener nebulizer, collision–reaction interface (CRI) and a three channel peristaltic pump, was used. The operating conditions are summarized in Table 2. Data acquisition in the steady-state mode with 10 s integration was used for the batch method and for the analysis of digests. On-line leaching was performed in time-resolved mode with three points per peak, one scan per replicate, a dwell time of 80 000 μs and 0.025 a.m.u. spacing.

Mitigation of spectroscopic interference. Use of the CRI was required to mitigate spectroscopic interference from polyatomic ions, in particular $^{40}Ar^{12}C^+$ and $^{35}Cl^{16}O^1H^+$ on $^{52}Cr^+$ and $^{40}Ar^{35}Cl^+$ on $^{75}As^+$. These interferences were especially prominent in artificial gastric juice, due to the presence of pepsin and HCl. The introduction of H_2 gas through a hollow skimmer cone effectively reduced these polyatomic interferences. The optimal H_2 gas flow rate was found using artificial gastric juice containing 10 μg L^{-1} Mn, which was used as a surrogate of Cr. The H_2 gas flow rate that maximized the signal ratio (m/z 55 over m/z 52) without unduly decreasing sensitivity at m/z 55 was selected. The auto-optimization function of the Varian 820MS Expert Software was then applied to the voltages on the ion optics while aspirating a gastric juice solution containing 20 μg L^{-1} of Sc, As and Y to maximize the signal-to-background ratio.

Table 2 ICPMS operating conditions (Varian 820MS)

Parameter	Setting
Plasma gas flow rate	Ar, 18.0 L min^{-1}
Auxiliary gas flow rate	Ar, 1.80 L min^{-1}
Nebulizer flow rate	Ar, 1.05 L min^{-1}
Sample uptake rate	0.8 mL min^{-1}
Sampling position	6.2 mm
Ar sheath gas flow rate	0.06 L min^{-1}
RF power	1.40 kW
CRI skimmer gas flow rate	H_2, 85 mL min^{-1}
Monitored signals	$^{45}Sc^+$, $^{52,53}Cr^+$, $^{54,56,57}Fe^+$, $^{58,60,62}Ni^+$, $^{63,65}Cu^+$, $^{64,66}Zn^+$, $^{75}As^+$, $^{89}Y^+$, $^{110,112-114}Cd^+$, $^{115}In^+$, $^{206-208}Pb^+$

Analysis methods

Batch method. Approximately 1 g of wheat sample was placed in a 50 mL falcon tube with artificial saliva. The test tube was placed in a shaker for 10 min at human body temperature (37 °C). The tube was then centrifuged for 15 min at 4100 rpm and 3 °C. The supernatant was decanted and filtered, with the filtrate finally collected in a clean bottle (high-density polyethylene). The procedure was then repeated with artificial gastric juice and finally intestinal juice, each shaken for 2 h. Quantitative analysis of the supernatants was carried out by ICPMS with external calibration using five matrix-matched standard solutions. Internal standardization was performed through the on-line addition of 5 μg L^{-1} Sc, Y and In through a Y connector.

On-line leaching method. A 0.4 g aliquot of wheat sample was rolled in glass wool and then placed into a 5 cm long polytetrafluoroethylene (PTFE) tube having 5/16-in outer diameter and 1/4-in inner diameter, thereby resulting in a "mini-column". A glass wool plug was placed at each end to trap the sample in the column throughout the experiment. A blank mini-column was prepared by only packing glass wool. In each case, the mini-column was connected to the nebulizer of the ICPMS instrument. Then, artificial reagents (saliva, gastric and intestinal juices) were sequentially pumped using the peristaltic pump.

The mini-column, gastro-intestinal reagents and standard solutions were all maintained at 37 °C using a thermostatically controlled water bath (Haake, Berlin, Germany). The leaching time was set to 5 min for each reagent after ensuring that the analyte signal returned to baseline within this time.

Flow injection was used to perform a four-point external calibration, where standard solutions and a blank (prepared in each leaching reagent) were injected through a 100 μL injection loop connected to a universal automatic actuator (Anachem Ltd., Luton, England). Quantification was based on peak area. No internal standardization was used with on-line leaching.

Verification of mass balance. To verify mass balance, 2.5 mL of concentrated HNO_3 and 0.5 mL of H_2O_2 were used to digest the residue in digestion vessels (Savillex) at 50 °C for 1 h. Each digest was then diluted to 25 mL with DDW. A blank was prepared using the same procedure but without sample. Standard solutions were prepared in the same matrix. Internal standardization was performed through the on-line addition of 5 μg L^{-1} Sc, Y and In through a Y connector.

Data processing

All analyses were carried out in five replicates. Blank subtraction was systematically performed prior to any further data processing.

Statistical analysis. The data analysis function in Microsoft Office Excel 2016 was used for statistical analysis. Grubbs test was performed at the 95% confidence level to identify outliers, which were then removed. A Student's *t*-test at the 95% confidence level was done to compare measured concentrations to certified values (the uncertainties on the latter for Cr and Cu were reported by Ihnat[22]). When comparing the two methods, an *F* test was first done to determine if there was a significant difference in precision between the two sets of results and then the appropriate Student's *t*-test was applied.

Figures of merit. The limit of detection (LOD) and the limit of quantification (LOQ) were respectively estimated as 3 times and 10 times the standard deviation of each reagent blank signal ($n = 10$) divided by the slope of calibration curve (*i.e.*, sensitivity). The resulting solution concentrations were converted to concentrations in the solid by multiplying by the volume of reagent used for leaching and then dividing by 0.4 g sample mass. Method precision was expressed as the % relative standard deviation (RSD) for 3–5 replicate measurements of hard red spring wheat flour SRM 8437.

Results and discussion

Validation of the on-line leaching method

The accuracy of the on-line leaching method was checked by applying it to a certified reference material, NIST SRM 8437 hard red spring wheat flour, prior to applying the method to samples. The results are summarized in Table 3, along with LODs and LOQs in each reagent. For each of the analytes with certified values, the sum of the amount leached by each of the three reagents and that remaining in the residues agrees with the certified value at the 95% confidence level according to a Student's *t*-test. The accuracy of the method for the determination of As was previously verified by applying it to NIST SRM 1568a rice flour.[14] In the case of Ni, Cd and Pb, the fact that similar results were in general obtained by the on-line and batch methods (as discussed later) suggests that the method is accurate for those elements as well. The increase to 0.4 g (instead of 0.2–0.25 g previously used)[13–15] permitted determination of the analytes with a precision of 9–23%, which is an improvement because % RSD could previously be as high as 33%.[14,15]

Total concentration of elements in wheat

Fig. 2 demonstrates that the total concentrations of elements vary between wheat samples, which may reflect different sources (*i.e.* soil, irrigation water, fertilizers or industrial contaminations).[23] However, some elements, such as Cr, As and Pb,

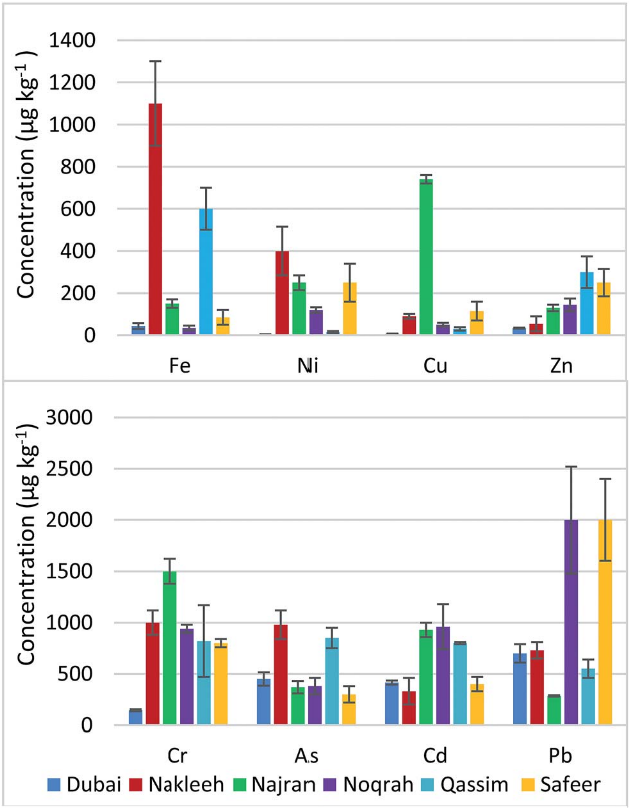

Fig. 2 Concentration (μg kg^{-1}) (mean ± standard deviation, $n = 5$) of selected elements in different wheat samples following total digestion.

Table 3 Concentrations in μg kg^{-1} (mean ± standard deviation) obtained by the on-line leaching method for SRM 8437 as well as the precision of the method along with the limits of detection and of quantification in each leaching reagent

	Saliva (μg kg^{-1})			Gastric juice (μg kg^{-1})			Intestinal juice (μg kg^{-1})					
Element	LOD	LOQ	Measured	LOD	LOQ	Measured	LOD	LOQ	Measured	Sum of leached and left in residue	Certified (information) value	RSD (%)
Cr ($n = 3$)	4	13	13 ± 4	0.5	1.5	2.0 ± 0.1	0.2	1	3.0 ± 0.2	21 ± 4	26 ± 12 (ref. 22)	19
Fe ($n = 4$)	13	40	740 ± 90	30	100	3500 ± 260	9	30	520 ± 190	34 000 ± 3000	31 000 ± 6000	9
Ni ($n = 5$)	5	16	125 ± 25	1	3	4 ± 0.9	0.1	0.4	1.2 ± 0.8	130 ± 20	(200)	15
Cu ($n = 3$)	6	20	60 ± 15	10	50	2008 ± 570	20	50	650 ± 90	2910 ± 670	2010 ± 490 (ref. 22)	23
Zn ($n = 3$)	10	40	780 ± 150	6	21	20 ± 4	20	80	260 ± 40	10 140 ± 1300	10 600 ± 1400	12
As ($n = 5$)	3	10	33 ± 2	2	5	6 ± 2	1.3	4	5.0 ± 0.2	40 ± 4		10
Cd ($n = 4$)	1	4	20 ± 1	10	40	70 ± 5	6	20	20 ± 3	120 ± 15	(20)	12
Pb ($n = 5$)	1	4	5 ± 1	3	11	11 ± 5	1	4	6 ± 1	26 ± 5		19

show similar distribution patterns in the central region (Safeer and Noqrah). Furthermore, the 40 ± 1 μg kg^{-1} Fe reported for wheat from the North agrees with the 44 ± 14 μg kg^{-1} Fe found in Dubai in this study.

Interestingly, the concentrations of Pb are significantly higher in the central region (Safeer and Noqrah), *i.e.*, 2000 ± 520 μg kg^{-1} and 2000 ± 400 μg kg^{-1} respectively. A similar Pb content of 2000 ± 100 μg kg^{-1} in the central area wheat was reported by Ali and Al-Qahtani[7] In contrast, the same study reported higher Pb concentrations of 4000 ± 100 μg kg^{-1} and 3000 ± 100 μg kg^{-1} in North area wheat and South area wheat, respectively,[2] than measured in this work. They indicate a heavily polluted area, Saudi Arabia being known to have significant Pb contamination.[5] In fact, the levels of Cr, As, Cd and Pb are of concern. Each variety of wheat thus requires bio-accessibility studies for realistic risk assessment.

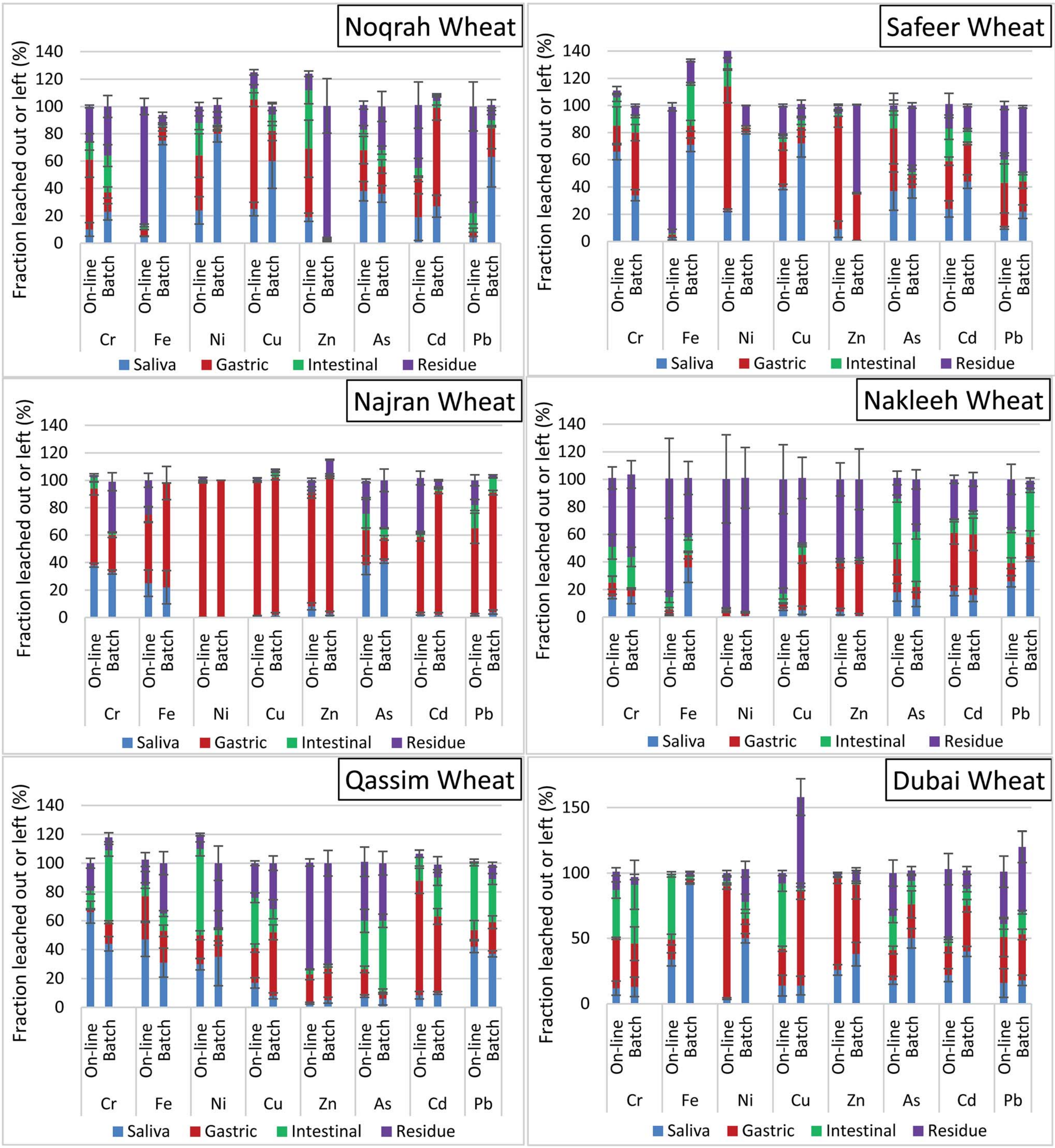

Fig. 3 Fraction of the concentration of different elements determined by total digestion that is bio-accessible (*i.e.* released by gastro-intestinal reagents) or is left in the residue following leaching by the three reagents.

Bio-accessibility of elements in wheat

Fig. 3 shows the percentage of each element's total concentration that is bio-accessible (*i.e.* released by each gastro-intestinal reagent) or left in the final residue. Bio-accessibility of an element clearly depends on the type of wheat. Huge differences can exist. For example, Ni is not bio-accessible from Nakleeh wheat but is entirely released by gastric juice from Najran wheat. In general, the low pH, coupled with pepsin complexation, can considerably enhance metal solubility in the gastric phase.[13,25–27] The highest bio-accessibilities in gastric juice are observed for Ni (20–100%), Cu (30–100%), Zn (30–80%) and Cd (22–80%), whereas Pb (5–35%), Fe (10–50%), Cr (10–45%) and As (10–30%) were not released as much by this reagent.

As can be seen in Fig. 3, saliva released a significant amount of different elements from all samples. This mobilisation generally occurred within a minute of leaching with saliva, indicating that the elements were either adsorbed on the sample or weakly bound to it.[13] It likely arises from the physicochemical properties of phosphate in saliva, which can substitute arsenate for example.[13,24] That is why phosphate-containing reagents are being used to extract As from dietary supplement, soil and sediment.[26–29]

In contrast, artificial intestinal juice generally released a smaller quantity of elements (if at all) than other gastrointestinal reagents. A similar observation was made with wheat bread.[13] It is well known that pancreatin in intestinal juice may lead to the formation of insoluble complexes, which thus reduces bio-accessibility.[9]

However, when looking at the sum of the bio-accessibilities in the three reagents, several elements are essentially 100% bio-accessible. While this is fine for essential elements, such as Ni and Cu in Najran wheat, it is concerning for toxic elements, like Cd and Pb in Qassim wheat. Even 60% bio-accessibility, such as for Pb in Safeer wheat (with 2000 μg kg^{-1} Pb!) can be a concern, especially for children.

Comparison of the results obtained by the on-line and batch methods

The on-line leaching method was modified in this study compared to previous iterations, where 0.4 g (instead of 0.2 or 0.25 g) powdered wheat sample was packed into a PTFE tube. The advantage of increasing the amount of sample in the mini-column is that the resulting signal is higher, which in turn leads to more reproducible results. Moreover, changing the length and diameter of the mini-column made sample preparation easier and minimized problems of back-pressure compared to previous studies with rice and bread.[13–15]

In most cases, the sum of the bio-accessible fraction and that remaining in the residue agree with the measured total concentration, as determined following total digestion of the wheat, according to a Student's *t*-test at the 95% confidence level. Furthermore, with the exception of Fe, Zn, Cd and Pb in Noqrah wheat, both methods provided very similar bio-accessibility results, according to a Student's *t*-test at the 95% confidence level. This agreement is despite on-line leaching involving just 5 min with each reagent as opposed to 10 min for saliva and 2 h each for gastric and intestinal juices with the batch method. With on-line leaching, the dissolution equilibrium is shifted towards the product side due to continuous leaching with fresh reagent, which significantly shortens the leaching times.

In contrast, the batch method determines equilibrium concentrations in a closed system. The on-line method is also less susceptible to contamination due to less sample manipulation and consumes less reagent than the batch method. In addition to being more efficient than the batch method, it provides real-time information, while being faster in terms of extraction time per sample. On the other hand, the on-line method requires more ICPMS instrument time than the batch method, *i.e.*, 15 min per sample as opposed to less than 1 min, which increases the frequency of instrument maintenance. Furthermore, with on-line leaching, only one sample can be treated at a time, while several wheat samples can be processed in parallel with batch methods.[9–11]

Table 4 Number of cups of wheat corresponding to the PTDI level of some elements, based on bio-accessible concentrations and one cup of wheat weighing 300 g

	As		Cd		Pb	
Permissible level (mg kg^{-1} body weight)	0.5		0.2		0.2	
Weight of person	70 kg	15 kg	70 kg	15 kg	70 kg	15 kg
Dubai	254	50	126	30	90	20
Nakleeh	130	20	117	20	60	10
Najran	430	90	55	10	170	30
Noqrah	580	120	47	10	35	7
Qassim	262	50	36	5	65	10
Safeer	580	120	117	25	60	10

Implications for human consumption

Using the bio-accessible concentrations of elements in the different wheat samples and the provisional tolerable daily intake (PTDI) of the joint expert committee of the Food and Agriculture Organization and the World Health Organization,[30,31] the PTDI was converted into the number of cups of wheat that would be safe for consumers, assuming one cup of wheat weighing 300 g. A relatively small number of cups was obtained for As, Cd and Pb, which is summarised in Table 4. The smallest number of cups was obtained for Cd in Qassim wheat: 5 cups per day for a 15 kg child and 36 cups per day for a 70 kg adult. Children are at greater risk because of their small body weight.

Conclusions

A modified on-line leaching approach coupled with ICPMS was successfully employed to estimate the maximum bio-accessibilities of both potentially toxic and essential elements in different wheat samples. The results confirm the effectiveness of the on-line leaching approach in comparison to a conventional batch method: it provides similar results to the batch method in 15 min instead of 250 min. Furthermore, it also requires less time for sample preparation, minimizes contamination, as it is conducted in a closed system, and provides real-time data, which previously allowed the identification of two different sources of Pb in corn bran.[19] The results showed that the total concentration and bio-accessible fraction of elements in wheat can differ between regions in Saudi Arabia. The study revealed that several of the elements of concern, As, Cd and Pb in particular, were present at up to 1000–2000 μg kg^{-1}, with a significant portion being bio-accessible (up to 80% in the case of Cd). This is a concern, especially for children. Considering bio-accessibility is thus important for realistic risk assessment. Future work will look at the effect of cooking on bio-accessibilities, as wheat is not normally eaten raw. Speciation analysis of bio-accessible As and Cr should also be carried out to find out the portion that is in toxic form.

Conflicts of interest

There are no conflicts to declare.

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