ANALYSIS OF POTENTIALLY TOXIC BIOACCESSIBLE ELEMENTS IN LOCALLY AVAILABLE BREAD USING CONTINUOUS ON-LINE LEACHING COUPLED TO INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

By

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Abstract

A previously developed, efficient and simple on-line leaching method was used to assess the bioaccessible fraction of potentially toxic elements (Cr, As, Pb and Cd) in whole wheat brown and white bread samples. Artificial saliva, gastric juice and intestinal juice were successively pumped into a mini-column, packed with bread (maintained at 37°C) connected on-line to the nebulizer of an inductively coupled plasma mass spectrometry (ICP-MS) instrument equipped with a collision-reaction interface (CRI) to mitigate the impact of polyatomic interferences. In contrast to the conventional batch method to which it was compared, this approach provides real-time monitoring of potentially toxic elements that are continuously leached with 3 artificial gastro-intestinal reagents. Mass balance for both methods was verified at the 95% confidence level. Results obtained from the whole wheat brown and white bread showed that the majority of Cr, Pb and Cd was leached by gastric juice but, in contrast, the majority of As was leached by saliva. Results showed a higher total content for elements in whole wheat bread than in white bread but in contrast, there was a higher percentage of bioaccessible elements in white bread than in whole wheat bread. This difference may be attributed to the several food processing steps involved for white bread compared to whole wheat bread. Both the on-line and batch methods showed that 40% -98% of toxic elements in bread samples are bioaccessible. Hydrogen as the CRI gas was efficient at minimizing carbon- and chlorine-based polyatomic interferences.

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Chapter 1

Introduction

1.1 Toxic elements in bread

Bread, a globally consumed food item, is an excellent source of proteins, vitamins, minerals, fiber, and complex carbohydrates, which consist of three or more sugars linked together to form a chain (http://www.fitday.com/fitness-articles/nutrition/carbs/simple-vs-complex-carbohydrates.html#b). The manufacturing methods used for bread production, the agricultural practices of cereal cultivation, and the bioaccumulation (i.e. accumulation in various tissues of a living organism) of pollutants are all potential sources of contamination for heavy and toxic metals in bread (Soares, Vieira, & Bastos, 2009). A previous study indicated that wheat and other crops grown in soils contaminated with different toxic and heavy metals (i.e. Cr, As, Cd, etc.) contain significant amounts of these metals within the grains (Wang *et al*., 2002). Hence the necessity to analyse bread samples to determine both their nutritional value and their toxicological content. Different methods have thus been developed to determine the elemental content of bread and its main constituent, flour (**Table 1.1**).

In order to gain an understanding of the differences between brown bread and white bread, one must look at its basic constituents, wheat grains, which contain three distinguishable parts: bran, endosperm and germ (**Figure 1.1**). The bran, i.e. the husk on

the outside, constitutes 15% of the grain and is high in fiber as well as in vitamins and minerals such as B1, B2, B3, B6, Mg, Fe, Zn and P. The core is made up of the endosperm, which is over 80% of the grain, and consists of proteins and carbohydrates. The rest of the wheat grain consists of the germ and also contains a good amount of vitamins, minerals, fat and proteins (http://wholegrainnation.eatbetteramerica.com/facts/). Whole wheat flour includes all parts of the grain, i.e. bran, endosperm and germ, thus

Table 1.1 Some selected methods used to determine elemental content in bread and its principal constituent, wheat flour

Analyte	Matrix	Sample preparation	Detection method	Calibration strategy	Reference
Cr	Bread	Closed-vessel acid digestion (mixture of nitric acid and sulfuric acid)	ETAAS*	Method of standard addition	(Bratakos, Lazos, & Bratakos, 2002)
Cr	Bread	Closed-vessel acid digestion (HNO_3 and H_2O_2)	ETAAS	Matrix-matched external calibration	(Soares *et al.*, 2009)
Cr, Cd and Pb	Bread	Microwave digestion system	ETAAS	Method of standard addition	(Alberti-Fidanza, Burini, & Perriello, 2002)
Cr, Cd and Pb	Durum wheat flour	Microwave closed-vessel digestion	ICP-MS**	Matrix-matched external calibration and internal standardization	(Cubadda & Raggi, 2005)
Cr, Cd, As and Pb	Wheat flour	Acid digestion (HNO_3 and H_2O_2) inhigh pressure reactor.	ICP-MS	Matrix-matched external calibration and internal standardization	(Vrček & Vinković Vrček, 2012)

*Electrothermal atomic absorption spectrometry

** Inductively coupled plasma mass spectrometry

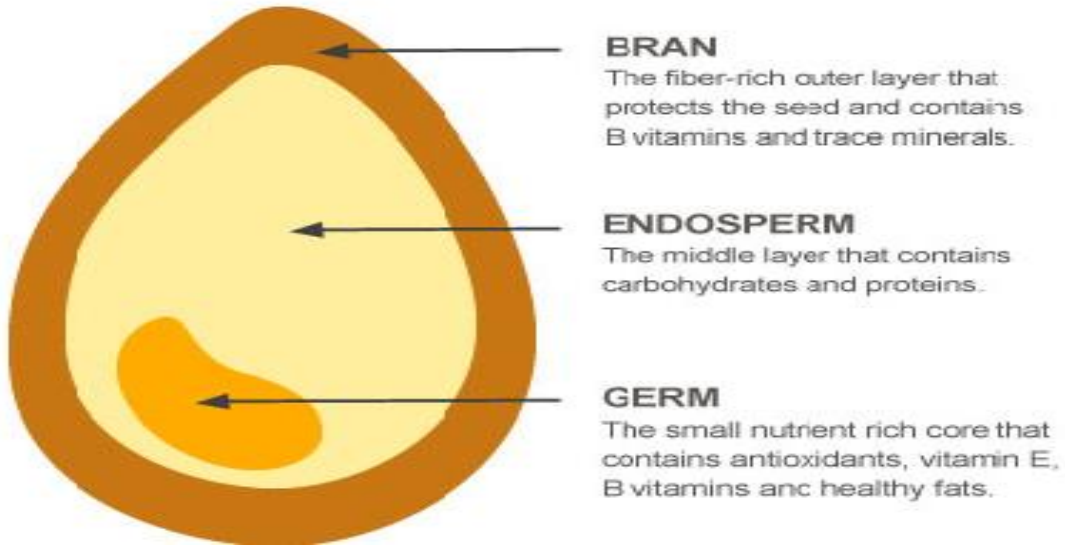

Figure 1.1 The anatomy of a whole wheat grain (http://wholegrainnation.eatbetteramerica.com/facts/)

resulting in brown bread that is more nutritious and healthier than white bread. Indeed, white flour is made up of only endosperm. The fiber-rich and nutritious parts of the wheat grain, i.e. bran and germ, are removed during processing. (http://wholegrainnation.eatbetteramerica.com/facts/). Enriched white flour is prepared through a series of processing steps, including the addition of different chemicals to bleach the flour as well as fortification with minerals and other nutrients that have been lost during processing. (http://wholegrainnation.eatbetteramerica.com/facts/).

Bread, like other food types, is a primary source of essential elements (e.g. Fe, Cu and Zn) and a significant means of exposure to toxic elements (e.g. As, Cd and Cr) for humans. It is important that the levels of these elements, both essential and toxic, be

determined by food industries both to implement regulatory standards and to evaluate long-term exposure risks. These regulatory standards are established by national governments and international organizations, such as the European Union, to facilitate world trade and improve the health of citizens of all nations. Most regulatory standards are accepted only after analytical procedures have been validated through inter-laboratory trials and have been shown to meet specified analytical criteria. In general, these standards dictate the maximum total concentration of elements and sometimes of specific species (such as inorganic As). Unfortunately, the potential hazardous effects of metal contamination cannot be estimated on the basis of the data from total metal assays. Indeed, all the toxic elements present in one's diet may not be available for absorption in the human gastro-intestinal system (Kulkarni *et al.*, 2007). Some foods may still be safe for human consumption even when they contain significant amounts of toxic elements, provided that these elements are not released in toxic amounts during gastro-intestinal digestion. The extent to which this occurs or not should thus be taken into account for adequate risk assessment of human exposure.

1.2 Bioavailability and bioaccessibility

The concepts of bioavailability and bioaccessibility are interpreted in various ways in the literature. For some authors, the two terms are even interchangeable at times (Caussy, 2003). In any case, they are predominantly important in quantifying the risks that are

associated with oral exposure to environmental contaminants (Wragg *et al*., 2011). In this chapter, bioavailability and bioaccessibility will be defined according to the context of

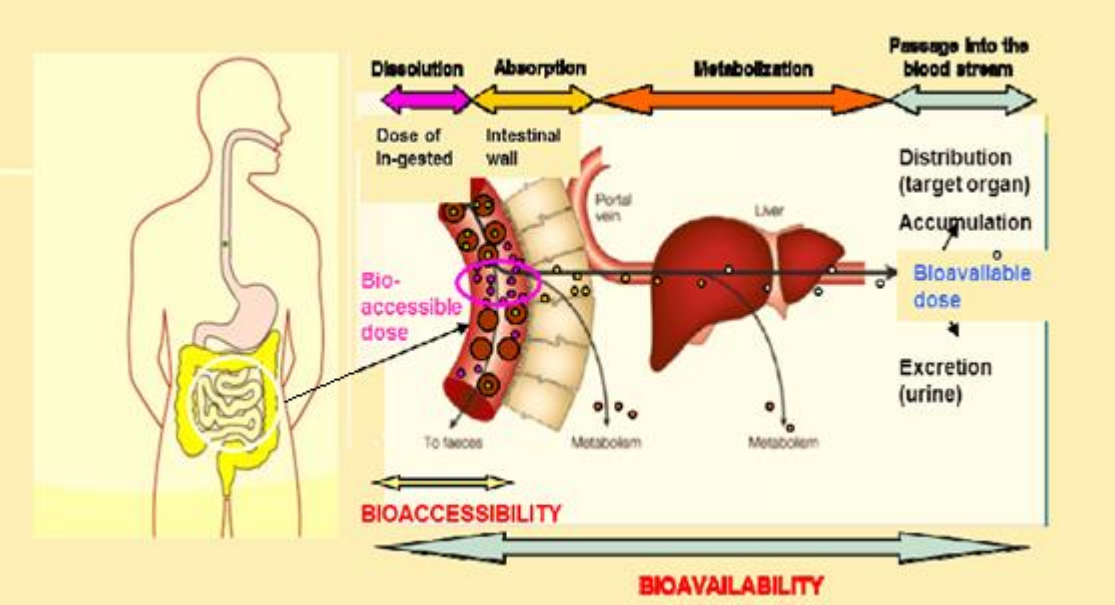

Figure 1.2 Schematic representation of bioavailability and bioaccessibility (Guigonnet-Sergent & Charissou, 2012)

human health risk assessment. The term bioavailability has been defined as the efficiency with which the elements are absorbed by the systemic circulation system and further exerts their toxic effects on target organs (Brandon *et al.*, 2006). The absorption, distribution, metabolisation and excretion processes that play a part in bioavailability are schematically represented in **Figure 1.2**. Conceptually, bioavailability can be viewed as the integral sum of three distinct processes consisting of: (1) the maximum fraction of the contaminant in food products that are leached to the liquid media from its matrix in the gastro-intestinal tract (bioaccessibility), (2) the fraction of bioaccessible species that is able to permeate across the cellular membrane by physiological processes (environmental availability) and (3) metabolism in the intestine and liver, which includes

internal processes, such as contaminant uptake, distribution, accumulation or exertion of toxic effects in targeted organ and excretion (toxicological bioavailability) (Oomen *et al.*, 2003; Rosende & Miró, 2013). This relationship can be written as follows:

$$BAv_i = BAc_i \times AB_i \times M_i \qquad \text{[Equation 1]}$$

where: BAv_i is the bioavailable fraction of substance i

BAc_i is the bioaccessible fraction of substance i

AB_i is the fraction of substance i absorbed through the intestinal wall

M_i is the fraction of substance i that is metabolized

The release of toxic species from ingested food in the gastrointestinal tract is an essential criterion for bioavailability of the toxic species in the body (Versantvoort *et al*., 2005). In other words, bioavailability fundamentally depends on bioaccessibility, which is defined as the fraction of the contaminant that is extracted from the matrix (dissolved) by saliva and digestive juices in the gastrointestinal tract and is therefore available for absorption (Ng *et al*., 2013). Thus, determining the bioaccessibility of a contaminant from its matrix can be seen as a potential indicator for the maximum bioavailability of the contaminant in the body and is therefore an important tool in risk assessment (Intawongse & Dean, 2006).

1.2.1 Factors affecting bioavailability and bioaccessibility

The human body is regulated by extremely complex mechanisms involving different physicochemical and physiological processes. Therefore, the bioavailability and

bioaccessibility of elements in food are affected by several factors, such as: the food matrix, the gastro-intestinal environment, individual characteristics of consumers (e.g., age, health status, body weight, etc.), when food is consumed, and the history of previous food consumption (McClements *et al*., 2008; Moreda-Piñeiro *et al.*, 2011). For example, the gastric emptying rate is slightly decreased in people over the age of 70 years, which is attributed to slower digestive processes (Horowitz *et al.*, 1991). Furthermore, the chemical form of elements found in food and certain other components of the food matrix can have a significant role in determining bioavailability and bioaccessibility. For instance, Se is toxic (especially when present as selenite and selenate) in large amounts but, in trace amounts, is an essential nutrient for humans and animals. According to the US Food and Nutrition Board, the recommended dietary allowance is 55 µg Se/day for both sexes, whereas the tolerable upper intake level is 400 µg Se /day in the USA (Pedrero & Madrid, 2009). The different chemical forms of Se are absorbed and metabolized differently in the human body. In general, organic compounds, e. g. selenomethionine, are more bioavailable than inorganic compounds such as selenite (Fairweather-Tait, 1997; Thomson, 2004). Moreover, certain food components not only affect the extraction kinetics of elements from the food but also the absorption kinetics of analyte in the gastrointestinal system (Sensoy, 2013). For example, vitamins E and A have been shown to increase Se bioavailability, whereas certain heavy metals and some dietary fibers can decrease it (Rayman, Infante, & Sargent, 2008; Reeves *et al*., 2007). In an experiment carried out by Laparra *et al.* using an *in vitro* gastrointestinal digestion model, a lower bioaccessible fraction of As was found in cooked edible seaweed than in

raw seaweed (Laparra *et al*., 2003). In contrast, As bioaccessibility was similar in cooked and uncooked long grain brown and white rice after simply washing the rice with water prior to cooking, as this removed a large percentage of the total fraction of bioaccessible As, Cu, V, Fe and Zn (Horner & Beauchemin, 2013).

The formulation of food products may result in the degradation of the complex food matrix, thereby increasing analyte bioaccessibility or may induce the formation of inhibitors or enhancers, which could decrease or increase bioaccessibility (Olivares *et al.*, 2001; Parada & Aguilera, 2007). In any case, the food matrix itself can affect bioaccessibility. For example, Cu was 100% bioaccessible from banana-based baby food while less than 50% was bioaccessible from meat-based baby food (do Nascimento da Silva *et al*., 2013). Even food contaminations with metals can have an impact on the bioavailability of essential metals (Schümann & Elsenhans, 2002). Furthermore, the human intestine is an intensely populated microbial ecosystem. As the principal role of the intestinal microflora is to salvage energy from carbohydrates, not digested in the upper gut, through fermentation (Salminen *et al*., 1998), it may enhance the elemental bioaccessibility/bioavailability.

1.2.2 Bioavailability and bioaccessibility determination methods

Various methods have been employed to evaluate the bioavailability of elements in food. Because there are several factors affecting bioavailability, as discussed in the previous

section, no single method has been identified as being suitable for all inorganic contaminants. Method selection is solely dependent on the contaminant of interest and the resources available to undertake bioavailability studies (Ng *et al*., 2013). Bioavailability of various elements is typically determined by *in vivo* models, which involve the administration of doses to either human volunteers or experimental animals such as rats .The main objectives of all bioavailability assays are identical, i.e. to obtain the best probable approximation of the amount of available toxins that may potentially cause harmful effects to the organisms (Caussy, 2003).

Generally, a response is monitored after feeding the subject with a certain dose of contaminants, and the changes in concentration are measured in end products as a function of time (Sensoy, 2013). This may include the determination of the contaminants in blood, target organs (liver and kidney), urine and faeces, or the measurement of urinary metabolites (e.g. methylated arsenicals), DNA adducts and enzyme induction (e.g. cytochrome P450 monooxygenases) (Ng *et al*., 2013). Although, human studies are the best approach for bioavailability studies, they are constrained by ethical considerations. Furthermore, the use of experimental animals is costly and time-consuming, not to mention that the selection of animals with similar gastrointestinal tract, metabolism, etc. as those of humans is somewhat difficult (Biehler *et al*., 2011). Moreover, the possible interaction of contaminants with other components in the food and the variability between individuals are weaknesses of *in vivo* studies (Parada &

Aguilera, 2007). Nevertheless, this approach is considered as the most accurate method when assessing the actual chemical risk to humans (Caussy, 2003).

An *in vitro* study offers a simple, inexpensive, rapid and convenient means of evaluating bioaccessibility (Kelley *et al*., 2002). A major advantage of this technique is that there is no ethical constraint. It can also improve reproducibility (Kong & Singh, 2008). The *in vitro* technique was originally designed to assess the bioaccessibility of iron from food in nutritional studies (Miller *et al.*, 1981). As bioavailability can only be smaller than or equal to bioaccessibility (Juhasz *et al.*, 2007), the environmental risk posed to humans from metals by ingesting soils and contaminated food can be assessed by evaluating their bioaccessibilities (Intawongse & Dean, 2006). The *in vitro* technique attempts to simulate the digestive process that takes place in two or three distinct regions (i.e. mouth, stomach and intestine) of the human body.

Various *in vitro* digestion designs, reviewed by (Intawongse & Dean, 2006), have been developed and applied for assessing the bioaccessibility of trace elements. The majority have been applied to soil samples (Oomen *et al.,* 2002) , a few of them to sea weeds (Laparra *et al.*, 2003, García-Sartal *et al.*, 2011) and one, each, to seafood (Laparra *et al*., 2007) and rice samples (He *et al.,* 2012). They can be sub-divided into two groups: static models involve the sequential exposure of samples to saliva, gastric and intestinal environments, whereas dynamic models simulate the gradual transit of a food mixture through the mimicked physiological conditions in the digestive tract (Oomen *et al*., 2002).

Oomen *et al.* (2002) described a multi-laboratory comparison and evaluation of the efficiencies of five different *in vitro* models (**Table 1.2**) for determining the

Table 1.2 Descriptions of selected *in vitro* models

	SBET [a] method	DIN method	in vitro digestion model	SHIME[b] method	TIM [c] method
Ref.	(Ruby *et al.*, 1996)	(Oomen *et al*., 2002; Rotard *et al*, 1995)	(Rodriguez *et al*., 1999)	(Laird *et al*., 2007)	(Oomen *et al*., 2002)
Amount of dry soil	1.0 g	2.0 g	0.6 g	10 g	10 g
Type of model	static stomach	static gastro-intestinal	static gastro-intestinal	static/dynamic gastro-intestinal	dynamic gastro-intestinal
Mechanical action	end-over-end rotation at 30 rpm	agitator 200 rpm	end-over-end rotation at 55 rpm	mechanical stirring at 150 rpm	Peristaltic movements
Volume of saliva and pH			9.0 mL 6.5		50 mL 5
Incubation time			5 min		5 min
Volume of gastric juice and pH	100 mL 1.5	100 mL 2.0	13.5 mL 1.1	25 mL 4.0	250 mL 5.0-2.0
Incubation time	1h	2h	2h	3h	Secretion of gastric content at 0.5 mL/min
Volume of intestinal juice and		100 mL	Duodenal juice: 27 mL; bile juice: 9 mL	Pancreatic fluid: 15 mL	3×70 mL (duodenum, jejunum and ileum)
pH		7.5	5.5	6.5	6.5; 6.8; 7.2
Incubation time		6 h	2 h	5 h	6 h

Detection method	ICP-AES[d] (As, Pb, Cd)	AAS[e] (As, Pb, Cd)	ICP-MS (As, Pb, Cd)	ICP-AES (As, Pb, Cd)	ICP-AES (Cd, Pb) and HG[f]-AAS (As)

a: Simple bioaccessibility extraction test; b: simulator of human intestinal microbial ecosystem in children; c: gastrointestinal model; d: ICP atomic emission spectrometry; e: atomic absorption spectrometry; f: hydride generation

bioaccessibility of As, Cd and Pb in soil, and demonstrated a wide range of bioaccessibility results. These *in vitro* digestion models are based on the processes occurring in the human gastrointestinal tract, which are summarized in **Table 1.3** (Caboche, 2009). They are all performed at body temperature (37°C). However, several models skip the oral cavity. Furthermore, none of these models provides real-time information. A number of comparative studies have indicated that bioaccessibility is largely dependent on the specific *in vitro* design (i.e. static or dynamic gastrointestinal), the actual contaminant under investigation and the sample matrix (Oomen *et al*., 2002, Torres-Escribano *et al*., 2011). Furthermore, other parameters such as gastric and

Table 1.3 Principal characteristics of the processes occurring in the human gastrointestinal tract

Compartments	Residence time	pH	Mechanical process	Secretion	Function
Oral cavity (Mouth)	Few seconds – few minutes	6.5	Mashing	Saliva	The crushing and saliva improves dissolution
Stomach	8-15 minutes(fasting) 1-2 h (feeding)	1-2 (fasting) 2-5 (feeding)	Mixing	Gastric juice	The acids dissolves the labile oxides, sulfates and carbonates

					thus freeing metals
Intestine					
Duodenum	0.5 – 0.75 h	4 - 5.5	Peristaltic motion	Intestinal juice Bile Pancreatic juice	Absorption of the contaminants freed from the matrix
Jejunum	1.5 – 2.0 h	5.5-7.0			
Ileum	5.0 – 7.0 h	7.0 – 7.5			

intestinal pH, food constituents, residence time and particle size also have a significant impact on bioaccessibility (Intawongse & Dean, 2006). In fact, from the comparison of five models in **Table 1.2**, the gastric pH was identified as the root cause for the difference in bioaccessibility results. The pH for maximum bioaccessibility was found to be 1.5-2.0, while the lowest bioaccessibility was observed at the highest pH (pH=4) (Oomen *et al*., 2002). The low pH of the stomach strongly favors an increase in the soluble fraction of metals, leading to higher bioaccessibility while the near neutral pH in the intestine results in metal precipitation (Grøn & Andersen, 2003). Waisberg *et al.* (2004) studied the effect of pH on the bioaccessibility of Cd from lettuce and confirmed that it decreased progressively as the pH increased from 1 to 6.5.(Waisberg *et al*., 2004) because the higher pH inhibits the dissolution of potentially bioaccessible metals (Wragg & Cave, 2002). A recent study compared the bioaccessibility of 24 inorganic elements obtained by 14 different laboratories using 17 different *in vitro* tests on the same soil reference material (NIST 2710) and demonstrated a negative correlation with pH for all elements (Koch *et al.,* 2013).

Most of the batch *in vitro* methods mentioned in **Table 1.2** ignore the leaching action of saliva, focusing on only the gastro-intestinal compartments, even though saliva has been shown to have significant leaching capability on food (Leufroy *et al*., 2012; Horner & Beauchemin, 2013). Although the procedures associated with batch models are relatively simple, they do suffer from some critical weaknesses: they do not provide kinetic information on the leaching process; they are extremely susceptible to contamination because they necessitate relatively large amounts of sample manipulation, which take time and are tedious; and they lack the natural dynamism that occurs in human digestion (Horner & Beauchemin, 2012). Moreover, metal re-adsorption and the re-distribution of extracted species may occur due to continuous contact and equilibrium between the solid and leaching agent, so that bioaccessibility may be underestimated (Miró *et al*., 2005; Rosende, Miró, & Cerdà, 2010).

Few batch dynamic models (SHIME and TIM as shown in **Table 1.2**) exist, which simulate the actual dynamic *in vivo* mechanism of the gastro-intestinal tract in human digestion. To date, the TIM dynamic model is the only fully characterized system, which consists of four computer-controlled chambers mimicking the conditions of the stomach, duodenum, jejunum and ileum (Minekus *et al*., 1995; Minekus *et al*., 1999; Torres-Escribano *et al.*, 2011). A continuous flow dialysis system has also been developed for assessing the mobility and fractionation of elements in soil samples (Arkasuwan, Siripinyanond & Shiowatana, 2011; Shiowatana *et al*., 2006). However, the experimental procedures associated with these dynamic models suffer from technological complexity,

high operating cost and large time consumption while only providing leaching data at punctual points in time. As a result, they cannot be used for routine risk assessment.

This is in contrast to a simple continuous on-line leaching method, which was originally developed to assess the mobility and fractionation of elements from soils and sediments (Beauchemin, Kyser, & Chipley, 2002) and was also successfully applied to the determination of bioaccessible Zn and Pb in corn bran, as well as As in seafood and rice Chu & Beauchemin, 2004; Dufailly *et al*., 2008; Horner & Beauchemin, 2012, 2013). It has distinct advantages over conventional batch models: it involves shorter sample preparation time, minimal sample manipulation and thus reduced contamination, while providing real-time information on the leaching kinetics of the metals released (Beauchemin *et al*., 2002). Furthermore, the dissolution equilibrium is driven to the right, due to the continuous pumping of fresh gastro-intestinal fluid through the sample, resulting in the release of maximum amounts of toxic elements from the food sample, in the absence of bacterial action in the gut and of potential synergistic effects from other food and beverage consumed along with bread (Horner & Beauchemin, 2013).

The literature shows that many studies have been carried out to categorize the levels of essential as well as toxic elements in food products to ensure food safety. As can be seen in **Table 1.2**, different analytical techniques were used. In recent years, ICP-MS has become a very popular and common tool for the routine analysis of food samples (Leblanc *et al.*, 2005) because it has some distinct advantages over other existing

techniques, such as great sensitivity, selectivity, simultaneous multi-element and isotope measurement capability coupled with very low detection limits (Ammann, 2007). Moreover, it also offers a wider linear dynamic range than flame AAS, ETAAS and ICP-AES, which allows the simultaneous determination of elements over a wide range of concentrations, down to ultra-trace level (Ammann, 2007). That is why ICP-MS is widely used for food analysis (Careri, Bianchi & Corradini, 2002). For instance, during the determination of sixteen elements in different types of food samples by ICP-MS, good agreement was obtained with certified values for CRM samples (Nardi *et al*., 2009).

1.3 Issues with inductively coupled plasma mass spectrometry

1.3.1 Spectroscopic interferences

While ICP-MS is a sensitive multi-elemental technique offering very low detection limits for many elements in the Periodic Table, a large linear dynamic range and simple spectra interpretation compared to those in ICP-AES (Beauchemin, 2010), the occurrence of spectroscopic interferences compromises instrumental selectivity. Spectroscopic interferences are caused by atomic and polyatomic ions with the same m/z as the ion of interest and originate from either the sample matrix or the plasma. They can hamper the trueness of some trace element analysis by ICP-MS (Taylor, 2001). Examples of spectroscopic interferences that may be encountered for selected analytes in this work are shown in **Table 1.4.** Several approaches have been implemented to tackle these interferences, such as using mathematical correction equations (Laborda *et al*., 2006), a

double-focusing sector field mass spectrometer (Ferguson & Houk, 2006), or a collision/reaction cell (Wolf, Morrison & Goldhaber, 2007).

However, each existing technique has its own benefits and inherent limitations. While double-focusing sector field ICP-MS may be the most straightforward for resolving several spectroscopic interferences, it has a high operational cost and complex design and

Table 1.4 Polyatomic interferences encountered for selected analytes during this work (May & Wiedmeyer, 1998)

Analyte	Interference
$^{52}Cr^{+}$	$^{40}Ar^{12}C^{+}$, $^{36}Ar^{16}O^{+}$, $^{38}Ar^{14}N^{+}$, $^{35}Cl^{16}O^{1}H^{+}$, $^{35}Cl^{17}O^{+}$
$^{53}Cr^{+}$	$^{37}Cl^{16}O^{+,}$ $^{35}Cl^{17}O^{1}H^{+}$, $^{35}Cl^{18}O^{+}$, $^{36}Ar^{16}O^{1}H^{+}$, $^{36}Ar^{17}O^{+}$, $^{38}Ar^{15}N^{+}$, $^{38}Ar^{14}N^{1}H^{+}$
$^{75}As^{+}$	$^{40}Ar^{35}Cl^{+}$, $^{38}Ar^{37}Cl^{+}$, $^{36}Ar^{38}Ar^{1}H^{+}$

is not always applicable for all matrices (Jakubowski, Moens & Vanhaecke, 1998; Laborda *et al*., 2006). Collision-reaction systems, such as collision-reaction cell (CRC), dynamic reaction cell (DRC) and octopole reaction system (ORS), where interferences from polyatomic ions are circumvented through reaction of analyte or polyatomic ions with different gases, have been frequently used for alleviating polyatomic interferences in ICP-MS (D'Ilio *et al*., 2011).Recently, a different device called collision-reaction interface (CRI) was introduced **(Figure 1.3**). It involves the introduction of reaction gas directly into hollow sampler and/or skimmer cones to overcome polyatomic ion

formation (Salazar *et al.*, 2011). At this point, the plasma still possesses a high temperature and ion density, which facilitate chemical reactions and collisions, leading to the efficient removal of interfering ions.

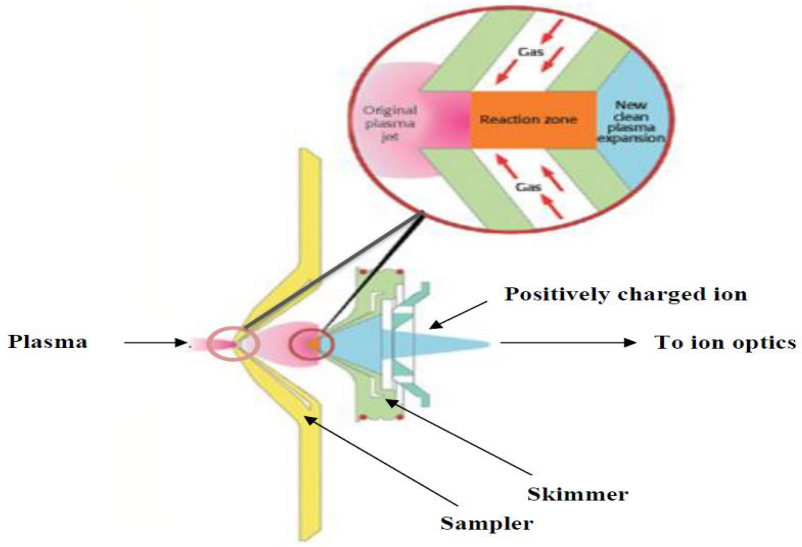

Figure 1.3 Diagrammatic illustration of the CRI system, where a collision/reaction gas is introduced through hollow cone(s). (http://www.varianinc.com/image/vimage/docs/applications/apps/icpms_an1.pdf)

In this thesis work, a quadrupole-based ICP-MS instrument equipped with a CRI was used for the determination of the bioaccessible fraction of toxic metals (Cr, As, Cd and Pb) from bread samples. When the CRI gas is injected through a groove inside the skimmer cone, it collides/reacts with plasma-related ions, thereby significantly reducing their velocities. Indeed, because polyatomic ions are larger than analyte ions, they have a higher probability of colliding with the CRI gas, which reduces their velocity. Once

polyatomic ions have lost a significant amount of kinetic energy, they become easier to divert off their route to the ion optics and the mass analyser. In general, introducing a CRI gas through the skimmer cone attenuates spectroscopic interferences more efficiently than through the sampler cone. While He and H_2 are most commonly used in CRI, H_2 is more effective than He because of its higher reactivity. **Table 1.5** summarizes some applications of H_2 as an effective reaction gas to minimize major polyatomic interferences on selected analytes. Indeed, using H_2 as CRI gas was reported to efficiently curtail most of the carbon-argon or chlorine-argon based polyatomic interferences (Bianchi *et al*., 2012; Leufroy *et al.*, 2011; Xing & Beauchemin, 2010). However, the CRI gas can also affect analyte ions, in particular their focusing. Therefore, a compromise CRI gas flow rate is vital in order to curtail interferences without jeopardizing instrumental sensitivity for analyte measurement (Varian 810/820MS customer training manual, 2009).

Table 1.5 Examples of application of H_2 as a reaction gas in ICP-MS to mitigate polyatomic interferences

Reaction system	Analyte	Interfering species	Reference
Hexapole	$^{56}Fe^+$, $^{57}Fe^+$	$^{40}Ar^{16}O^+$, $^{40}Ar^{16}O^1H^+$	Arnold, Harvey & Weiss, 2008
ORS	$^{40}Ca^+$, $^{44}Ca^+$, $^{55}Mn^+$, $^{56}Fe^+$,	$^{40}Ar^+$, $^{12}C^{16}O_2^+$, $^{40}Ar^{14}N^1H^+$, $40Ar^{16}O^+$,	Lear *et al.*, 2012
Hexapole	$^{78}Se^+$, $^{80}Se^+$	$^{38}Ar^{40}Ar^+$ $^{40}Ar^{40}Ar^+$	Marchante-Gayón *et al*., 2001
CRI	$^{52}Cr^+$, $^{53}Cr^+$, $^{75}As^+$	$^{40}Ar^{12}C^+$,$^{35}Cl^{16}O^1H^+$, $^{37}Cl^{16}O^+$, $^{40}Ar^{35}Cl^+$	Bianchi *et al*., 2012; Horner & Beauchemin, 2012; Xing & Beauchemin, 2010

1.3.2 Non-spectroscopic interferences

Non-spectroscopic interferences are considered just as problematic as spectroscopic interferences and will result in a suppression or enhancement of the analyte signal. The suppression/enhancement is due to the influence of various factors, from sample introduction to analyte ionization and detection. These effects can be divided into two categories: 1) effects arising from dissolved and undissolved solids in sample solution (i.e. physical effects); 2) suppression or enhancement effects arising in the plasma or within the mass spectrometer. For instance, as the total concentration of solids in solution increases beyond 0.2% m/v or 2 mg/mL, significant analyte signal drift may result with time, due to the deposition of solids on the orifice of sampler cone, which gradually reduce the sampler orifice diameter (Jarvis & Williams, 1998).

The degree of suppression or enhancement is influenced by the identity and absolute amount of matrix element, rather than by the relative amount of matrix to analyze (Liu & Beauchemin, 2006). However, there is still no clear explanation for all the observed matrix effects. The most common explanation is that matrix effects may arise from a combination of a shift in the atom-ion equilibrium and space charge effects within the mass spectrometer. In general, for a specific matrix element, matrix effects are more severe for lighter mass analytes, and heavy matrix elements cause the most severe matrix effects (Tan & Horlick, 1987). Also, matrix effects depend on the matrix concentration, with the effects getting more significant as matrix concentration increases. Other factors,

such as ionization potential, also have some impact on the resultant matrix effect. For example, easily ionized elements in the matrix can shift the ionization processes of other analytes by increasing the electron density, which may in turn induce analyte signal suppression (Hanselman *et al.*, 1994).

Several approaches have been taken to mitigate matrix effects. The simplest way is sample dilution, if the detection limit for the analyte is low enough and the analyte concentration is high enough to still enable analyte detection following dilution. The flow injection strategy has been used as an efficient method to reduce matrix effects because of dispersion resulting in on-line dilution of sample (Hywel & Evans, 1991). Some calibration strategies are used to compensate for matrix effects, such as matrix-matched external calibration, standard additions, internal standardization and isotope dilution analysis. If the matrices of the standard solutions and samples are exactly matched, quantification by external calibration should be possible because the same non-spectroscopic interferences should affect all of these solutions. In practice, matrix-matching is often difficult for real samples whose matrix is complex or unknown. Internal standardization is another useful technique to alleviate matrix effect. This involves the on-line or off-line addition of an appropriate internal standard to both the sample and standard solutions. For this approach to be effective, the internal standard should have a mass and first ionization potential similar to those of the analyte. It should not be present in the samples and should be affected in a similar manner as the analyte by the matrix.

Isotope dilution involves the measurement of the change in isotope signal ratio after adding a known quantity of analyte enriched in one isotope (it is often considered as the ideal internal standard). As the two isotopes have a very small mass difference and are subject to the same matrix, isotope dilution is ideal for overcoming matrix effects (Beauchemin & Specht, 1997) and is considered as an absolute quantification method, providing results with high precision and accuracy. If enriched isotopes are not available, the analyte to be determined is monoisotopic or the matrix is too complicated for matrix-matching, then the method of standard addition is the best choice, where the sample is sub-divided and spiked with increasing known amounts of analyte. In this way, the analyte in the unspiked and spiked samples is subject to the same matrix, and any matrix effect will be effectively corrected. However, it is time-consuming, as a preliminary estimation of the analyte concentration is required in order to obtain the most accurate results.

1.4 Thesis Objectives

The objective of this thesis work was to develop a relatively quick risk assessment method for some potentially toxic elements (Cr, As, Cd and Pb) in bread. To this end, the bioaccessibility of these elements was determined in different locally available whole wheat and white bread samples using the recently developed continuous on-line leaching dynamic *in vitro* method coupled to a quadrupole-based ICP-MS detector. The results were compared with those obtained using a conventional batch method to ensure that they were similar, as the ultimate goal was to obtain similar results as with the batch method but in a much shorter time. Mass balance was checked for both methods. In order to alleviate polyatomic interferences, optimization of the hydrogen CRI gas flow rate was carried out so as to improve the limits of detection for Cr and As in gastro-intestinal matrices, especially gastric juice, which results in significant spectroscopic interferences with those two analytes. Finally, the total concentrations and bioaccessible concentrations were compared with the available food safety regulations to assess the risk to human health.

Chapter 2

Experimental

2.1 Instrumentation

The research was conducted on a Varian 820MS (Varian Inc., Australia nowadays produced by Bruker Corp., Billerica, MA, USA) quadrupole-based ICP-MS instrument equipped with a CRI. The sample introduction system (illustrated in **Figure 2.1**) consists of a MicroMist concentric nebulizer (Glass Expansion, Victoria, Australia) fitted into a Peltier-cooled Scott double-pass spray chamber (SCP Science, Quebec, Canada) maintained at 0°C and a three channel peristaltic pump. A double off-axis quadrupole and a 90 ^{0} ion mirror (shown in **Figure 2.2**) are respectively used for ion extraction and ion focusing, which result in high sensitivity and low background.

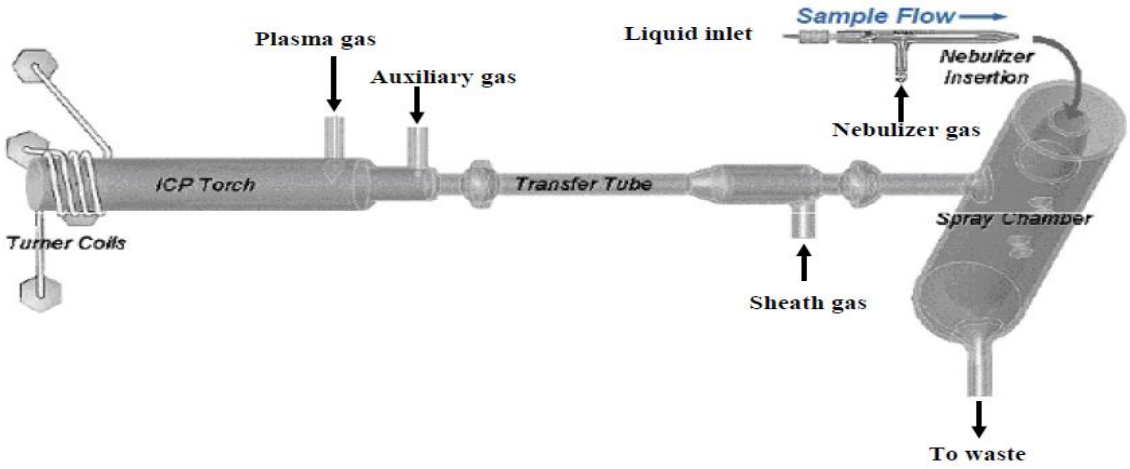

Figure 2.1 Diagram of Varian 820-MS sample introduction system (Varian 810/820MS customer training manual, 2009)

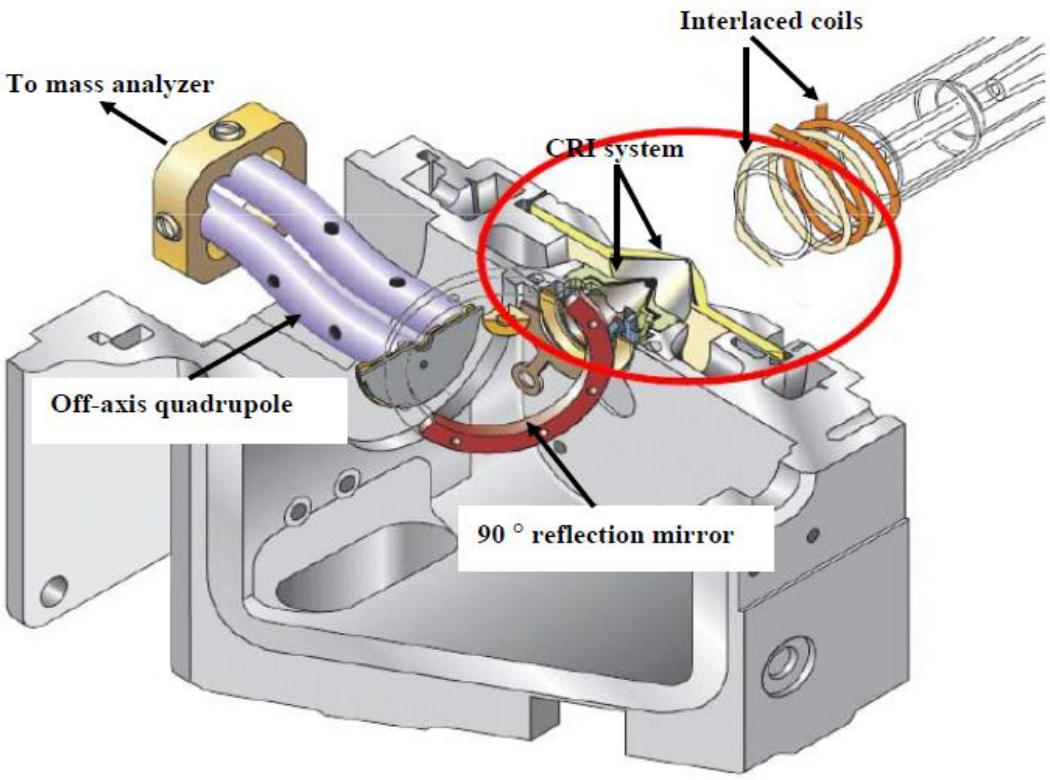

Figure 2.2 Spatial arrangement of the interlaced coils, the CRI system, the 90-degree reflection mirror and the off-axis quadrupole (http://www.varianinc.com/image/vimage/docs/products/spectr/icpms/brochure/si-0231.pdf)

Data acquisition during on-line leaching was performed in time-resolved mode with three points per peak, one scan per replicate, a dwell time of 80,000 ms and 0.025 amu spacing. Direct nebulization was done in steady-state mode with 10-s integration for the analysis of batch leachates and sample digests.

2.1.1 Optimization of CRI conditions to mitigate potential interferences

Optimization of the CRI gas flow rate is required, as too low a flow rate will not effectively mitigate spectroscopic interferences whereas too high a flow rate can impair the instrumental sensitivity. A compromise CRI gas flow rate must be found that effectively reduces the polyatomic interferences while minimizing the loss in instrumental sensitivity. In order to conduct a multi-elemental analysis of bread, a multivariate optimization of the H_2 CRI gas flow rate and other instrumental parameters (shown in **Table 2.1**) was carried out using Minitab 16 statistical software (Minitab Inc.) for the determination of Cr, As, Cd and Pb in artificial gastric juice, as gastric juice contains higher concentration of carbon and chloride than other gastrointestinal reagents and will thus give rise to higher spectroscopic interference on Cr and As.

The resulting compromise CRI H_2 gas flow rate was further validated by comparing the interfering signal at m/z = 52 to the signal of Mn^+ (m/z = 55), which was used as a surrogate element that is close in mass-to-charge (m/z) ratio to the target analyte. A gastric juice solution containing 10 μg L^{-1} Mn was aspirated and the signal ratio of m/z 52 over m/z 55 was continuously monitored while adjusting the CRI H_2 flow rate. As the CRI gas flow rate was increased, the signals at both m/z 52 and m/z 55 decreased, but that at m/z 52 (from polyatomic interferences) was reduced to a greater extent than that of Mn, resulting in an improved signal-to-background ratio. However, as the CRI gas flow rate was further increased, the ratio reached a maximum value and then decreased

Table 2.1 Parameters optimized using Minitab 16 software for gastric juice matrix

Parameter	Selected	Range
H_2 CRI gas flow rate (ml/L)	80	0 - 100
Ar nebulizer gas flow rate (L/min)	1.05	0.7 - 1.2
Sampling depth (mm)	6.2	5 - 7
RF power (kW)	1.40	1.3 - 1.7
Ar sheath gas flow rate (L/min)	0.06	0.0 - 0.1

(Xing & Beauchemin, 2010). The CRI H_2 gas flow rate corresponding to the maximum ratio was 80 mL/min, which was adopted for the remainder of this work. At this optimized CRI H_2 gas flow rate, the auto-optimization function in the Varian ICP-MS Expert software was applied to the voltages on the ion optics while aspirating a gastric juice solution containing 20 µg/L of Sc, As and Y. Torch alignment was performed daily using a tuning solution containing 5 µg/L of Be, Mg, Co, In, Ce, Pb and Ba in 2% nitric acid solution that was prepared by dilution of a 10 mg/L Varian tuning solution. The resulting instrumental conditions are summarized in **Table 2.2**.

2.2 Reagents

Artificial gastrointestinal juices were prepared according to USP XXIII, with one exception: no digestion enzyme was added to saliva. Artificial saliva was prepared with

6.8 g of KH_2PO_4 (ACS grade; Fisher Scientific, NJ, USA), 77 mL of 0.2 mol L^{-1} NaOH (ACS grade; BioShop, Burlington, Canada), diluting to 1 L using doubly deionized

Table 2.2 Instrumental parameters used for the analysis of bread

Parameter	Optimal value
Ar plasma gas flow rate (L /min)	18
Ar auxiliary gas flow rate (L /min)	1.8
Ar sheath gas flow rate (L/min)	0.06
Ar nebulizer gas flow rate (L/min)	1.05
Sampling depth (mm)	6.2
RF power (kW)	1.40
CRI skimmer gas and flow rate (mL/min)	H_2, 80
CRI sampler gas flow rate	Off
Monitored signals	$^{52}Cr^+$, $^{53}Cr^+$, $^{75}As^+$, $^{110}Cd^+$, $^{112}Cd^+$,$^{113}Cd^+$, $^{114}Cd^+$, $^{206}Pb^+$, $^{207}Pb^+$, $^{208}Pb^+$ $^{115}In^+$
First extraction lens (V)	-44
Second extraction lens (V)	-161
Third extraction lens (V)	-225
Corner lens (V)	-216
Mirror lens left (V)	40
Mirror lens right (V)	30
Mirror lens bottom (V)	33
Entrance lens (V)	-2
Fringe bias (V)	-2.6
Entrance plate (V)	-32
Pole bias (V)	0.0

water (DDW) (18.2 MΩ cm^{-1}) and finally adjusting the pH to 6.5 using 0.2 mol L^{-1} NaOH. All DDW used in this work was purified using an Arium Pro UV|DI water purification system (Sartorius Stedim Biotech, Göttingen, Germany). Artificial gastric juice was prepared by mixing 2.0 g of NaCl (ACS grade; BioShop, Burlington, Canada), 3.2 g of pepsin (Sigma-Aldrich, Oakville, Canada), 7.0 mL of sub-boiled HCl (ACS grade; Fisher Scientific, Ottawa, Canada), and diluting to 1 L (pH=1.2) using DDW. Artificial intestinal fluid was prepared by adding 6.8 g of KH_2PO_4, 10 g of pancreatin (which is a mixture of amylase, lipase and protease) (Sigma-Aldrich, St. Louis, USA), 77 mL of 0.2 mol L^{-1} NaOH, diluting to 1 L using DDW and finally adjusting the pH to 6.8 with 0.2M NaOH solution. For the digestion of residues, sub-boiled HNO_3 (ACS grade; Fisher Scientific, Ottawa, Canada) and H_2O_2 (J.T. Baker, Phillipsburg, USA) were used. All HNO_3 and HCl were purified prior to use with a DST-1000 sub-boiling distillation system (Savillex, Minnetonka, USA). Multi-element standard solutions were prepared in each leaching matrix using 1000 mg L^{-1} mono-element solutions (SCP Science, Baie d'Urfé, Québec, Canada).

2.3 Sample preparation

A loaf each of whole wheat and white breads (Dempster brand) was purchased from local grocery stores in Kingston, Ontario, Canada, representing the food as normally obtained by the average costumer. Soft and crust (hard) parts of each bread were separated and chopped with a stainless knife into small portions, placed in clean paper, and then dried at room temperature. One representative portion of each bread sample (about 10 g) was

ground to fine powder using porcelain mortar and pestle and stored in plastic bags (Zip lock) at room temperature for further experimental use.

2.4 Mini-column preparation

Glass wool was used for packing sample into mini-columns. It was soaked in 10% (v/v) nitric acid solution overnight and then washed with DDW. The glass wool was then washed with artificial saliva and finally air-dried. Aliquots of 0.25-0.30 g of bread samples were wrapped in glass wool and then inserted into a 10-cm long PTFE tube having, 3/15-in outer diameter and 1/8-in inner diameter, thereby resulting in a "mini-column". In addition, a glass wool plug was inserted at both ends of each mini-column in order to trap the sample in the mini-column. A blank mini-column was also prepared by simply packing glass wool in a mini-column without any sample.

2.5 Continuous on-line leaching set-up

A schematic diagram of the experimental set-up for on-line leaching is included in **Figure 2.3**. The continuous on-line leaching method was mostly as described in a previous study (Horner & Beauchemin, 2013). Artificial reagents (saliva, gastric and intestinal juices) were sequentially pumped into the mini-column containing food sample maintained at human body temperature (37˚C), using a water bath (Haake, Berlin, Germany) and the elements released were continuously monitored by ICP-MS (Chu & Beauchemin, 2004). The leaching time was set to 5 minutes for saliva and initially 1 hour

each for gastric and intestinal juices. However, the analyte signal for gastric juice returned to base line within 15 minutes and almost no, if any, peak was observed for intestinal juice. Therefore, the final leaching time was 15 minutes for gastric and 5

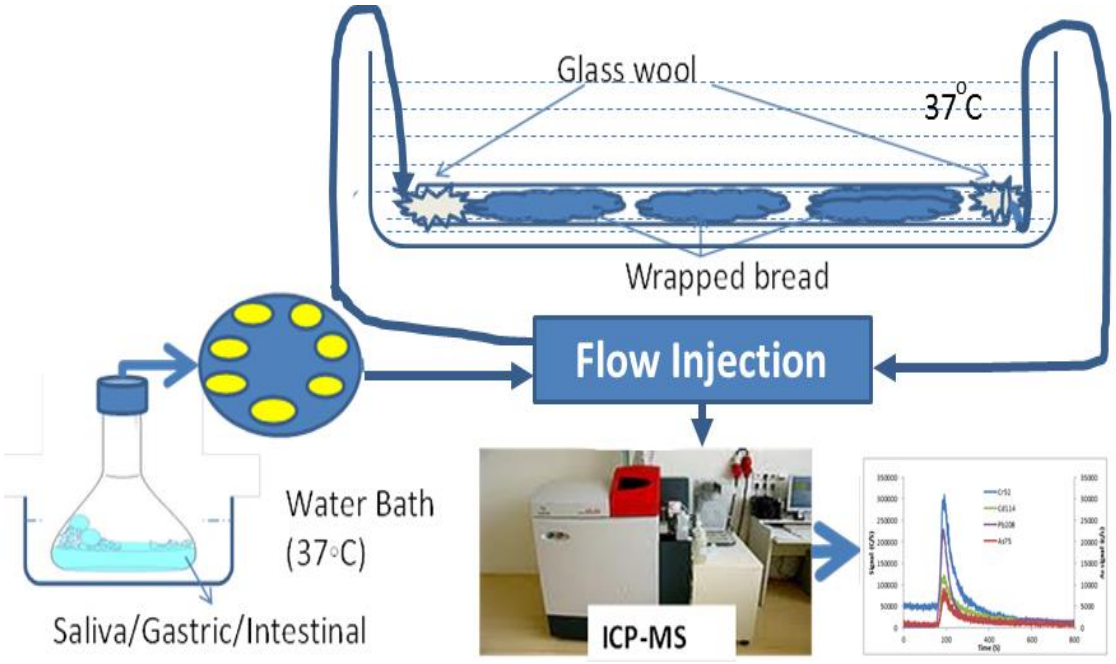

Figure 2.3 Schematic diagram of the experimental set-up used for on-line leaching

minutes for intestinal juice. The on-line leaching method continuously provides real-time monitoring of leaching kinetics for analytes as a function of time.

External calibration was done using flow injection, where standard solutions and blank (prepared in each leaching reagent) were injected through a 100-µL injection loop connected to a universal automatic actuator (Anachem Ltd., Luton, England). For the quantification, a four-point calibration curve was constructed of peak area versus the absolute analyte content (calculated as 100 µL × concentration of standard injected in

µg/L), and linear regression analysis performed using the data analysis function in Microsoft Office Excel 2010, to obtain the equation of the line of best fit, which was then applied to find sample concentration. As no drift was observed upon analyzing a test standard solution after every three samples in the analysis sequence, no internal standardization was used for on-line leaching.

2.6 Batch method

Figure 2.4 shows a flow chart of the batch method. About 1 g of bread sample was placed in a falcon tube and 6 mL of artificial saliva was added. The falcon tube was then put in an automatic shaker for 10 minutes at human body temperature ($37\ ^{0}$C), and then centrifuged 15 minutes at 4100 rpm and 3^{0}C to minimize further interaction of food material with the reagent. The supernatant was decanted off, filtered carefully and the filtrate was collected in a clean high density polyethylene (HDPE) bottle for further ICP-MS analysis. The procedure was repeated using gastric and intestinal juices each for 2 hours in order to mimic the *in vivo* digestion environment. The supernatant was quantitatively analyzed by direct nebulization with external calibration using 5 matrix-matched standard solutions and a reagent blank prepared daily. Internal standardization was performed by on-line addition of 5-μgL^{-1} In solution via a Y connector.

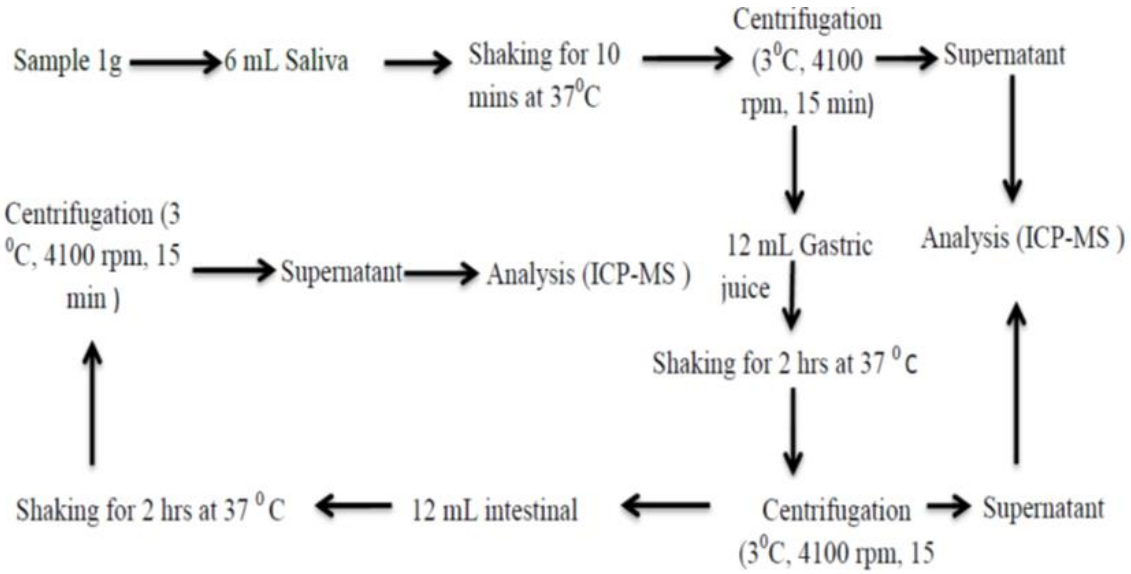

Figure 2.4 Schematic representation of the batch leaching method

2.7 Mass balance

A mass balance was performed for both methods, which required analysis of the leachates and residues in order to verify that the sum of the total bioaccessible mass and that remaining in the residue was equal to the total mass of analyte as determined by digestion of the sample. The residues were digested with 2.5 mL of sub-boiled HNO_3 and 0.75 mL of H_2O_2 in a digestion vessel (Savillex), with heating at 50 ^{0}C on a hot plate for 2 hours. The resulting solution was diluted to 25 mL with DDW prior to ICP-MS analysis. For the total sample digestion, 1-g bread aliquots were digested via the same procedure. Analysis of all the digests was carried out by matrix-matched external calibration. Again, 5 µg L^{-1} In internal standard was added on-line via a Y-connector.

Chapter 3

Method Characterization

3.1 Assessment of H_2 CRI to minimize argon-carbon and argon-chlorine based polyatomic interferences

The efficiency of the optimal H_2 CRI gas flow rate obtained using Minitab 16 was evaluated using a blank gastric juice solution containing 10 µg/L Mn, while monitoring Mn at m/z 55, as a surrogate element for Cr, and the background at m/z 52. As can be seen in **Table 3.1**, a very low 55/52 signal intensity ratio and a significant high background signal intensity at m/z 52 was obtained without CRI. This is evidence that high $^{40}Ar^{12}C^{+}$ and/or $^{35}Cl^{16}O^{1}H^{+}$ signals originated from the gastric juice because it contains carbon and chloride from its components, pepsin enzyme (3.2 g/L) and (7.0 mL/L) HCl, respectively.

Table 3.1 Signal intensity at m/z 52 and m/z 55 for gastric juice spiked with 10 µg/L Mn using different CRI modes

H_2 flow rate (mL/min)	m/z 52 (c/s)	%RSD (n=10)	m/z 55 (c/s)	%RSD (n=10)	55/52	Relative m/z 52 intensity (%)	Relative m/z 55 intensity (%)	Detection limit (µg/L)
0	1219000	1.5	3820000	1.1	3.13	100	100	1
50	289400	3.9	2168000	0.92	6.97	24	56	0.2
80	60600	3.5	1249000	0.80	20.6	5	33	0.09
100	23200	5.8	389600	1.2	14.3	2	10	0.4

As can be seen in **Table 3.1**, even a low CRI H_2 gas flow rate (50 mL/min) introduced through the skimmer cone can significantly reduce the background signal, around 4-fold for a matrix containing carbon and chlorine. A significantly lower background was obtained at a H_2 CRI flow rate of 100 mL/min from the gastric juice, but analyte signal sensitivity was also concurrently reduced. A comparatively higher signal ratio of m/z 52 over m/z 55 and the best detection limit were obtained at 80 mL/min. Thus, this condition was chosen for subsequent experiments. These results confirm the feasibility of H_2 gas introduced through the skimmer cone to overcome the positive interference caused by $^{40}Ar^{12}C^{+}$ and $^{35}Cl^{16}O^{1}H^{+}$ on ^{52}Cr signal.

3.2 Analytical characteristics

3.2.1 Detection limits and sensitivity

The optimal instrumental conditions shown in **Table 2.2** in Chapter 2 were used to determine the analytical characteristics, i.e. detection limit (DL), sensitivity and precision, for both the batch and on-line leaching methods. The DL was calculated as three times the standard deviation of the blank count rate (3σ, n =10) divided by the corresponding sensitivity, sensitivity being the slope of the calibration curve. The linear range of these methods was 0.3 ng/mL to 100 ng/mL, and the square of the correlation coefficient was better than 0.997 over the investigated concentration range. **Table 3.2** compares the detection limits and sensitivities for different analytes in different matrices obtained for both batch and on-line leaching methods.

Table 3.2 Detection limit (3σ, n = 10) (µg/L) and sensitivity (cps/µg/L) (± standard deviation, n = 4-5) for different analytes in different matrix for batch and on-line leaching methods

Method		Batch			Total digestion	On-line		
Matrix		Saliva	Gastric	Intestinal	10% HNO_3	Saliva	Gastric	Intestinal
Cr	DL	2	1	1	1	1	1	1
	Sensitivity	4580 ± 120	7100 ± 100	7400 ± 200	11800 ± 250	6100 ± 100	7700 ± 100	7100 ± 100
As	DL	0.2	0.4	0.2	0.2	0.3	0.4	0.4
	Sensitivity	750 ± 10	450 ±10	900 ± 10	760 ± 5	500 ± 10	450 ± 130	950 ± 50
Cd	DL	0.03	0.04	0.03	0.03	0.04	0.02	0.02
	Sensitivity	3460 ± 160	5950 ± 50	5700 ± 100	13600 ± 50	2850 ± 20	7800 ± 250	5750 ± 150
Pb	DL	0.5	0.4	0.5	0.03	0.6	0.5	0.5
	Sensitivity	4400 ± 100	7700 ± 100	7000 ± 100	74300 ± 700	11100 ± 600	13800 ± 300	15000 ± 100

Similar detection limits were obtained for all analytes regardless of the method used. However, the on-line leaching method offers a minimal amount of sample manipulation and the whole process is carried out in a closed system, which minimizes the possibilities of contamination. Furthermore, with on-line leaching, the analytes released are immediately swept towards the plasma in a minute amount of reagent whereas the analytes released in the batch method are diluted by the whole volume of reagent.

3.2.2 Reproducibility of the methods

The reproducibility of both the on-line and batch methods was expressed using the relative standard deviation (% RSD) of at least five replicate measurements of the sample. **Table 3.3** compares the reproducibility of the on-line and batch methods. When processing data from the on-line leaching method, the variation in peak area of the transient signal was used to calculate the %RSD. This was done for different analytes in saliva and gastric juice, as negligible amounts of analyte were released in the intestinal juice. In the case of the batch method, the variation in steady-state signal was utilized. A very similar trend in % RSD was observed for both methods even though a significantly larger amount of sample was analysed in the batch method. All samples were prepared from the same bread slice.

Table 3.3 Reproducibility of the on-line leaching and batch methods for different analytes in saliva and gastric juice

Type of bread	Method	Reagent	Cr	As	Cd	Pb
			%RSD	%RSD	%RSD	%RSD
Brown soft	On-line (n=5)	Saliva	12	5	8	26
		Gastric juice	11	16	8	16
	Batch (n=5)	Saliva	10	5	8	23
		Gastric juice	11	4	9	21
Brown crust	On-line (n=5)	Saliva	11	22	11	30
		Gastric juice	14	18	21	28
	Batch (n=6)	Saliva	13	14	10	26
		Gastric juice	12	15	17	21
White soft	On-line (n= 6)	Saliva	21	20	13	15
		Gastric juice	12	14	13	14
	Batch (n=6)	Saliva	19	14	9	11
		Gastric juice	9	8	8	9
White crust	On-line (n=6)	Saliva	17	8	22	18
		Gastric juice	13	20	24	20
	Batch (n=6)	Saliva	13	11	7	12
		Gastric juice	16	17	12	21

Chapter 4

Application of the method to bread samples

This chapter highlights the application of a simple continuous on-line leaching method to assess the bioaccessibility of potentially toxic Cr, As, Cd and Pb from bread samples, with a comparison to the conventional batch method.

4.1 Total metal content analysis in bread

The soft (interior) and hard (crust) parts of each type of bread were analyzed separately to check for possible metal contamination from the baking pan. Indeed, Cr may be a major contaminant if the pan is made from stainless steel. Total digestion of each bread sample was first conducted to ensure that the total content of analytes was high enough to be detected. **Table 4.1** shows that not only was this the case but significantly higher concentrations of Cr, Cd and Pb were present in whole wheat bread as compared to white bread. Similar Cr contents of 280 ± 80 ng/g in whole wheat bread and only 70 ± 30 ng/g in white bread were reported by Bratakos *et al.* (2002). In contrast, Soares *et al.* (2009) reported a similar total Cr concentration of 51 ± 22 ng/g and 47 ± 20 ng/g in whole wheat and white bread respectively. These differences likely reflect differences in purity of the ingredients, grown in different geographical areas, in particular, flour and water, which are the main constituents of bread. **Table 4.2** shows the Cr concentration range in potable water and wheat flour found in the literature

Table 4.1 Absolute concentration (ng/g) (mean ± standard deviation, n = 6) of selected elements in bread following total digestion of sample and Student's t-test comparison at the 95% confidence level.

Sample		Cr	Student's t-test		As	Student's t-test		Cd	Student's t-test		Pb	Student's t-test	
			Found	Table		Found	Table		Found	Table		Found	Table
Whole wheat	Soft	250 ± 50	2.10	2.20	60 ± 10	1.09	2.22	90 ± 10	1.09	2.22	250 ± 40	0.76	2.17
	Crust	200 ± 30	2.10	2.20	55 ± 5	1.09	2.22	85 ± 5	1.09	2.22	270 ± 50	0.76	2.17
White	Soft	100 ± 10	1.73	2.17	50 ± 10	1.73	2.17	16 ± 1	2.19	2.22	100 ± 30	0.0	2.30
	Crust	90 ± 10	1.73	2.17	60 ± 10	1.73	2.17	18 ± 2	2.19	2.22	100 ± 10	0.0	2.30

Table 4.2 Chromium concentration in potable water and wheat flour found in literature

Sample	Chromium content	Reference
Durum wheat flour	20.0 – 31.5 (ng/g)	(Cubadda & Raggi, 2005)
Wheat flour	30.03 – 33.03 (µg/kg)	(Vrček & Vinković Vrček, 2012)
Wheat flour	20.0 – 31.5 (ng/g)	(González, Gallego, & Valcárcel, 1999)
Potable water	nd – 11.8 (µg/L)	(García *et.al* 1999)
Potable water	0.49 (µg/L)	(Xing & Beauchemin, 2010)
Potable water	0.3 – 4.3 (µg/L)	(Hugues *et al*. 1994.)

As can be seen from **Table 4.1,** the total amounts of Cr, Cd and Pb in whole wheat bread are significantly higher than in white bread, while a similar concentration of As was found in all bread samples. The bread fabrication process (such as preparing the dough) may also be a potential route of contamination, which would however affect both types of bread similarly. The similar amounts of elements present in the soft and crust part of the bread, within the 95% confidence interval, rule out metal contamination from the baking pan during the baking process itself. However, punctual metal contamination may still be possible when using a new baking pan.

4.2 Bioaccessible metal content in bread

4.2.1 On-line leaching profiles

Figures 4.1, 4.2, 4.3, and **4.4** show typical on-line leaching profiles for Cr at m/z = 52, As at m/z = 75, Cd at m/z = 114 and Pb at m/z = 208 in the whole wheat bread soft part with the different artificial gastro-intestinal fluids, along with blanks. All the analytes were released in a fairly similar fashion, within the first ninety seconds of saliva reaching the food sample. This is evidence that the elements are adsorbed/weakly bound to the bread sample and that saliva has significant leaching power so should be included in bioaccessibility studies, unlike previous studies (Oomen *et al*., 2002). Furthermore, saliva also serves to wet the food, which facilitates gastric juice digestion.

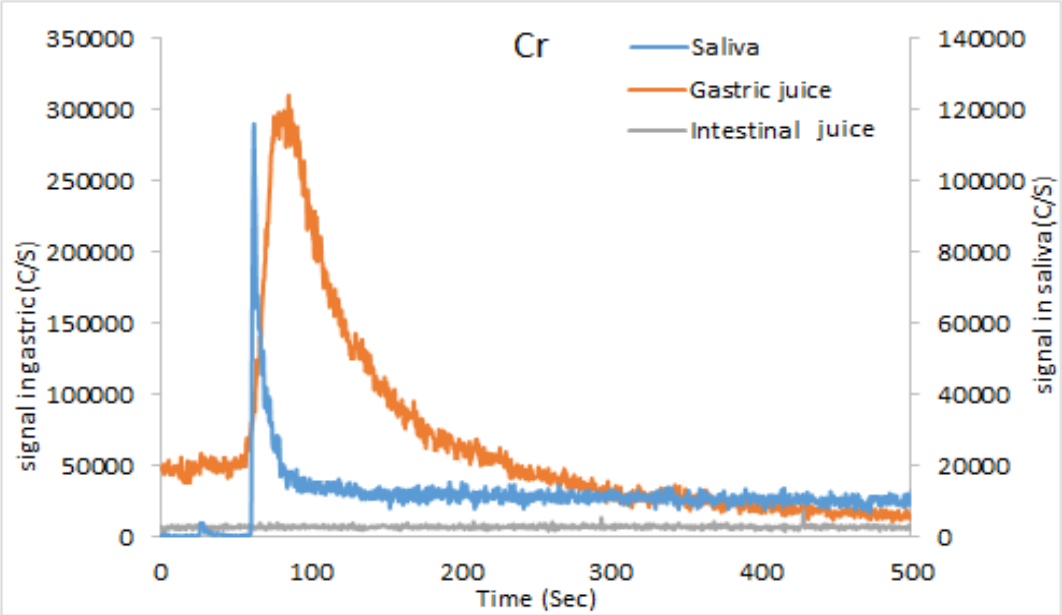

Figure 4.1 Raw on-line continuous leaching profiles for soft part of whole wheat brown bread, observed while monitoring $^{52}Cr^{+}$ by ICP-MS, using artificial gastro-intestinal fluids for leaching.

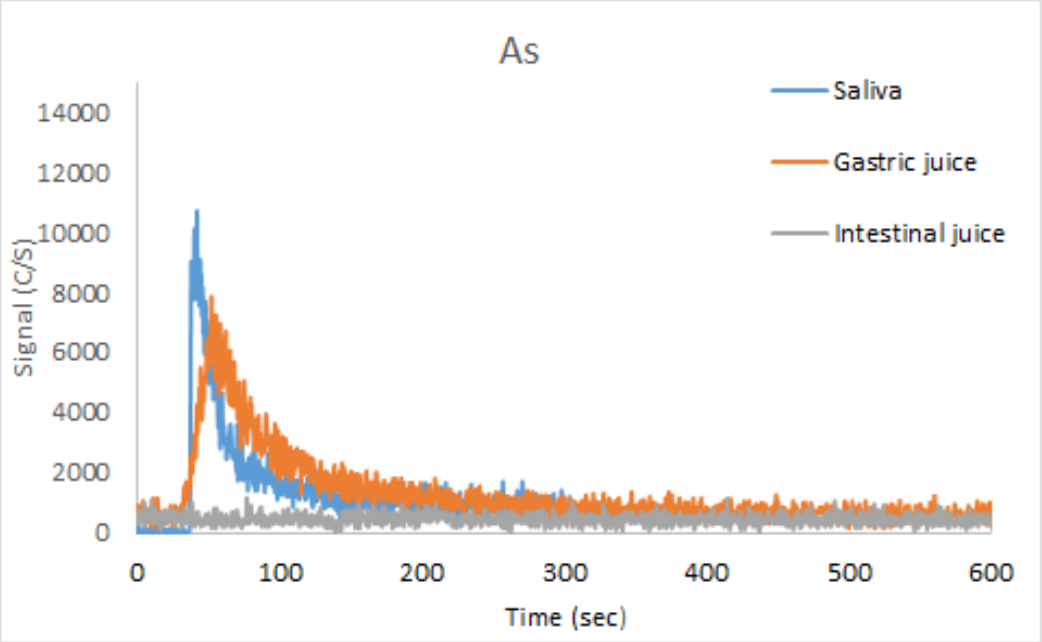

Figure 4.2 Raw on-line continuous leaching profiles of soft part of whole wheat brown bread, observed while monitoring $^{75}As^{+}$ by ICP-MS, using artificial gastro-intestinal fluids for leaching.

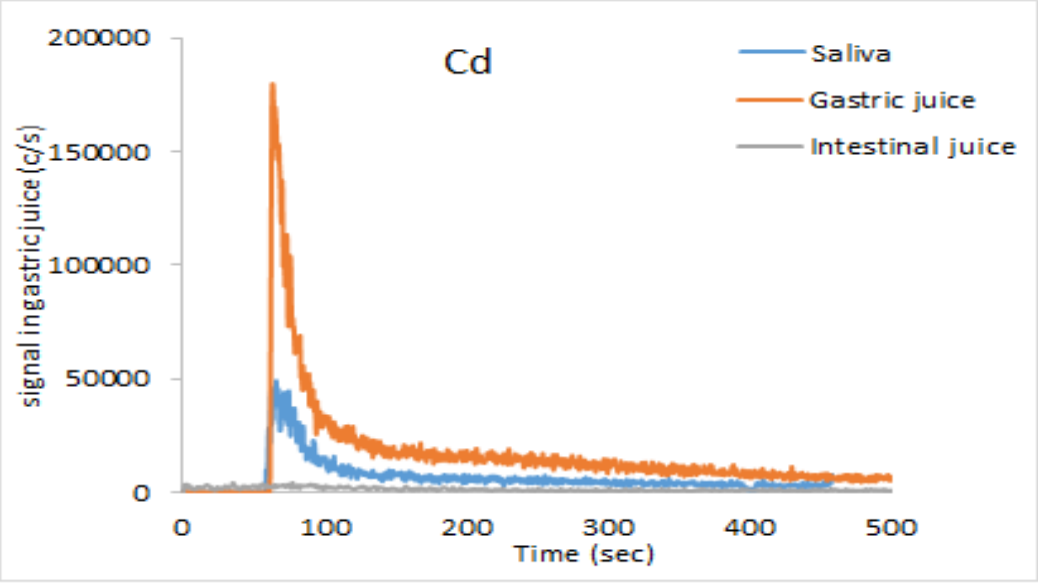

Figure 4.3 Raw on-line continuous leaching profiles of soft part of whole wheat brown

bread, while monitoring $^{114}Cd^{+}$ by ICP-MS using artificial gastro-intestinal fluids for leaching.

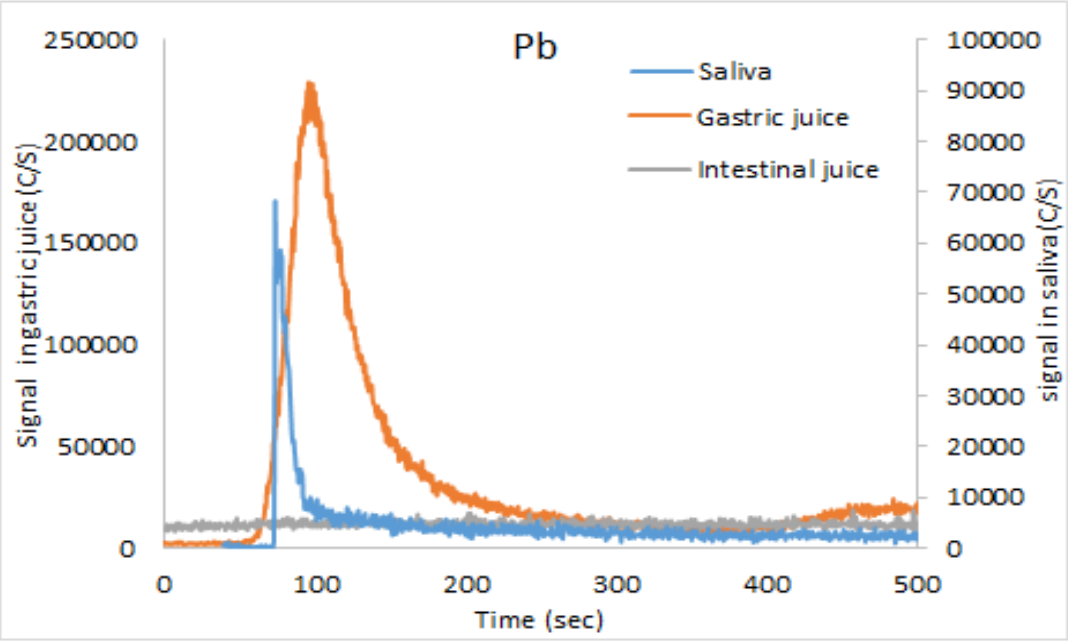

Figure 4.4 Raw on-line continuous leaching profiles of soft part of whole wheat brown bread, while monitoring $^{208}Pb^{+}$ by ICP-MS, using artificial gastro-intestinal fluids for leaching.

A different leaching behavior was observed when artificial gastric juice was continuously pumped through the bread sample, following leaching with saliva**.** As can be seen in **Figures 4.1**, **4.3** and **4.4,** the leaching profiles for Cr, Cd and Pb were much larger and broader than in saliva. On the other hand, the opposite was true for As, as a smaller peak was obtained in gastric juice than in saliva **(Figure 4.2).** Clearly, in gastric juice, more time is needed for the mobilization of Cr, Cd and Pb than in saliva. This is not surprising because it is analogous to digestion in the human stomach, where residence time for food

is roughly 2 hours. The more rapid mobilization of As is likely due to its lower concentration, even more so as most of it was released by saliva.

Comparison of the saliva and gastric juice leaching profiles reveals that gastric juice released more bioaccessible Cr, Pb and Cd than saliva. Possible metal complexation or chelation with amino acids present in pepsin enzyme and the low pH of gastric juice likely significantly increased metal solubility (Wragg *et al*., 2011; Intawongse & Dean, 2006). This is consistent with studies done on other sample types (Koch *et al*., 2013; Waisberg *et al*., 2004). In contrast, the larger amount of As released by saliva than by gastric juice is similar to what was previously reported for As in rice (Horner & Beauchemin, 2012, 2013). Therefore, the bioaccessible As is more weakly bound to the bread matrix than Cr, Pb and Cd. So, this continuous on-line leaching approach not only provides information on bioaccessibility, but also some specific information regarding the source of the elements in the sample (Chu & Beauchemin, 2004; Dufailly *et al*., 2008). In contrast, no detectable amounts of Cr, As, Pb and Cd were released by artificial intestinal juice (**Figures 4.1, 4.2, 4.3** and **4.4**). Hence, leaching with saliva and gastric juice was sufficient to release bioaccessible Cr, As, Pb and Cd from bread. Of course, this does not take into account potential synergistic effects from other food or beverage that would be consumed along with bread, or bacterial effects in the gut.

4.2.2 Comparison of total amounts of elements released by on-line and batch methods

Tables 4.3 and **4.4** show the mean total amounts of Cr, As, Pb and Cd leached by each of the three reagents and the amounts remaining in the residue, using continuous on-line leaching and a conventional batch method for the soft and hard part of whole wheat and white bread samples. In all cases, the sum of bioaccessible fraction and remaining amounts in the residue agrees with the measured total concentration, as determined by the total digestion of the bread, according to a Student's t-test at the 95% confidence level.

Table 4.5 shows that both methods provided total bioaccessibility results in agreement at the 95% confidence level according to a Student's t-test, except Pb (for which mass balance was not verified in the batch mode), even though the on-line leaching time for each of gastric and intestinal juices was just 15 minutes, compared to 2 hours for the batch method. Because the on-line method involves the continuous leaching of the sample with fresh reagent, the dissolution equilibrium is naturally shifted towards the product side, which in turn significantly shortens the leaching times. This may be the reason why the fraction of elements released by intestinal juice is significantly smaller with the on-line leaching method than with the batch method.

Table 4.3 Concentration in ng/g of leached and total Cr, As, Cd and Pb obtained by the on-leaching and batch for soft and crust part of whole wheat brown bread and Student's t-test comparison with expected value at the 95% confidence level.

Analyte	Sample	Leaching method	Saliva	Gastric juice	Intestinal fluid	Bioaccessible fraction	Bioaccessible fraction + residue	Expected	Student's t-test	
									Found	Table value
Cr	Whole wheat soft	On-line (n=5)	30 ± 4	70 ± 5	0.5 ± 0.1	100 ± 10	220 ± 20	250 ± 50	1.24	2.57
		Batch (n=5)	12 ± 3	90 ± 20	6 ± 3	110 ± 20	240 ± 30	250 ± 50	0.38	2.44
	Whole wheat crust	On-line (n=5)	35 ± 5	80 ± 10	2 ± 1	120 ± 10	230 ± 20	200 ± 30	1.86	2.36
		Batch (n=5)	30 ± 10	70 ± 10	10 ± 2	110 ± 10	200 ± 10	200 ± 30	0.19	2.26
As	Whole wheat soft	On-line (n=5)	16 ± 2	11 ± 2	0.10 ± 0.01	30 ± 3	50 ± 5	60 ± 10	2.00	2.44
		Batch (n=5)	20 ± 2	6 ±1	2 ± 1	30 ± 3	50 ± 10	60 ± 10	1.58	2.30
	Whole wheat crust	On-line (n=5)	25 ± 5	9 ± 2	0.3 ± 0.1	35 ± 5	60 ± 10	55 ± 5	1.00	2.44
		Batch (n=5)	15 ± 5	10 ± 3	7 ± 2	30 ± 10	50 ± 10	55 ± 5	1.00	2.44

Analyte	Sample	Leaching Method	Saliva	Gastric	Intestinal	Bioaccessible fraction	Bioaccessible fraction + residue	Expected	Student's t- test	
									Found value	Table
Cd	Whole wheat soft	On-line (n=5)	20 ± 2	40 ± 3	1.0 ± 0.1	60 ± 4	90 ± 10	90 ± 10	0.63	2.30
		Batch (n=5)	20 ± 4	40 ± 3	10 ± 1	70 ± 5	90 ± 10	90 ± 10	0.63	2.30
	Whole wheat crust	On-line (n=5)	20 ± 3	50 ± 10	2.5 ± 1.5	70 ± 10	90 ± 10	85 ± 5	1.00	2.44
		Batch (n=5)	15 ± 1	60 ± 5	0.3 ± 0.2	75 ± 5	80 ± 5	85 ± 5	1.58	2.30
Pb	Whole wheat soft	On-line (n=5)	25 ± 5	70 ± 10	0.30 ± 0.01	100 ± 10	270 ± 20	250 ± 40	1.00	2.44
		Batch (n=5)	660 ± 250	670 ± 170	n.d*.	1300 ± 400	1400 ± 400	250 ± 40	6.39	2.57
	Whole wheat crust	On-line (n=5)	15 ± 5	85 ± 25	2 ± 1	100 ± 25	200 ± 30	270 ± 50	2.68	2.44
		Batch (n=5)	60 ± 40	650 ± 200	45 ± 5	760 ± 250	1000 ± 300	270 ± 50	5.36	2.57

n.d*. = not detected

Table 4.4 Concentration in ng /g of leached and total Cr, As, Cd and Pb as obtained by the on-leaching and batch methods for soft and crust part of white bread and Student's t-test comparison with expected value at the 95% confidence level.

Analyte	Sample	Leaching method	Saliva	Gastric juice	Intestinal fluid	Bio-accessibe fraction	Bioaccessible fraction + residue	Expected	Student's t-test	
									Found	Table value
Cr	White soft	On-line (n=6)	30 ± 10	60 ± 10	0.10 ± 0.05	90 ± 10	110 ± 10	100 ± 10	1.73	2.22
		Batch (n=5)	25 ± 5	60 ± 10	5 ± 1	90 ± 10	110 ± 20	100 ± 10	1.00	2.44
	White crust	On-line (n=6)	10 ± 2	70 ± 10	0.10 ± 0.10	80 ± 10	100 ± 10	90 ± 10	1.73	2.22
		Batch (n=5)	10 ± 2	50 ± 10	10 ± 1	70 ± 10	100 ± 10	90 ± 10	1.58	2.30
As	White soft	On-line (n=6)	30 ± 10	13 ± 5	nd*	40 ± 10	60 ± 10	50 ± 10	1.73	2.22
		Batch (n=5)	25 ± 5	10 ± 1	8 ± 1	45 ± 5	60 ± 10	50 ± 10	1.58	2.30
	White crust	On-line (n=6)	30 ± 2	10 ± 3	0.30 ± 0.20	40 ± 4	70 ± 10	60 ± 10	1.73	2.22
		Batch (n=5)	20 ± 4	10 ± 2	5 ± 1	35 ± 5	65 ± 5	60 ± 10	1.00	2.44

Analyte	Sample	Leaching method	Saliva	Gastric juice	Intestinal fluid	Bio-accessibe fraction	Bioaccessible fraction + residue	Expected	Student's t-test	
									Found	Table value
Cd	White soft	On-line (n=6)	3.0 ± 0.4	10 ± 2	nd*	13 ± 2	14 ± 2	16 ± 1	2.19	2.30
		Batch (n=5)	2.5 ± 0.5	8 ± 1	0.5 ± 0.1	11 ± 1	16 ± 1	16 ± 1	1.65	2.22
	White crust	On-line (n=6)	3 ± 1	14 ± 4	nd*	17 ± 4	20 ± 4	18 ± 2	1.09	2.30
		Batch (n=5)	4 ± 1	11 ± 1	0.5 ± 0.2	15 ± 2	19 ± 1	18 ± 2	1.00	2.30
Pb	White soft	On-line (n=6)	30 ± 10	45 ± 15	nd*	75 ± 15	120 ± 30	100 ± 30	1.15	2.22
		Batch (n=5)	250 ± 50	300 ± 30	25 ± 5	580 ± 60	800 ± 100	100 ± 30	14.99	2.77
	White crust	On-line (n=6)	25 ± 5	60 ± 10	5±2	90 ± 10	110 ± 20	100 ± 10	1.09	2.36
		Batch (n=5)	30 ± 10	300 ± 200	40±10	400 ± 200	500 ± 300	100 ± 10	3.26	2.57

Table 4.5 Comparison of batch and on-line methods in terms of bioaccessible fraction of soft and crust parts of brown and white bread using Student's t-test at the 95% confidence level.

Analyte	Bread part	Leaching method	Whole wheat bread bioaccessible fraction	Student's t-test		White bread bioaccessible fraction	Student's t-test	
				Found	Table value		Found	Table value
Cr	soft	On-line	100 ± 10	1.00	2.44	90 ± 10	0.73	2.26
		Batch	110 ± 20			90 ± 10		
	crust	On-line	120 ± 10	1.58	2.30	80 ± 10	1.65	2.26
		Batch	110 ± 10			70 ± 10		
As	soft	On-line	30 ± 3	0.52	2.22	40 ± 10	1.01	2.44
		Batch	30 ± 3			45 ± 5		
	crust	On-line	35 ± 5	1.00	2.44	40 ± 4	1.80	2.30
		Batch	30 ± 10			35 ± 5		
Cd	soft	On-line	60 ± 5	3.16	2.30	13 ± 2	2.14	2.30
		Batch	70 ± 5			11 ± 1		
	crust	On-line	70 ± 10	1.00	2.44	17 ± 4	1.07	2.30
		Batch	75 ± 5			15 ± 2		
Pb	soft	On-line	100 ± 10	6.70	2.77	75 ± 15	19.88	2.44
		Batch	1300 ± 400			580 ± 60		
	crust	On-line	100 ± 25	5.87	2.77	90 ± 10	3.79	2.57
		Batch	760 ± 250			400 ± 200		

Figures 4.5 and **4.6** express the results of **Tables 4.5** as percentages of the total metal content in the soft and hard parts of bread, by batch and on-line methods. In **Figure 4.5**, 40 to 55% of Cr was bioaccessible from whole wheat bread using both the batch and on-line leaching methods. Over 50% of the As was bioaccessible with both on-line and batch methods for both parts of whole wheat bread. The Cd bio-accessibilities ranged from 60 to 80%. Nearly 40% of the Pb was bioaccessible in whole wheat bread by on-line leaching.

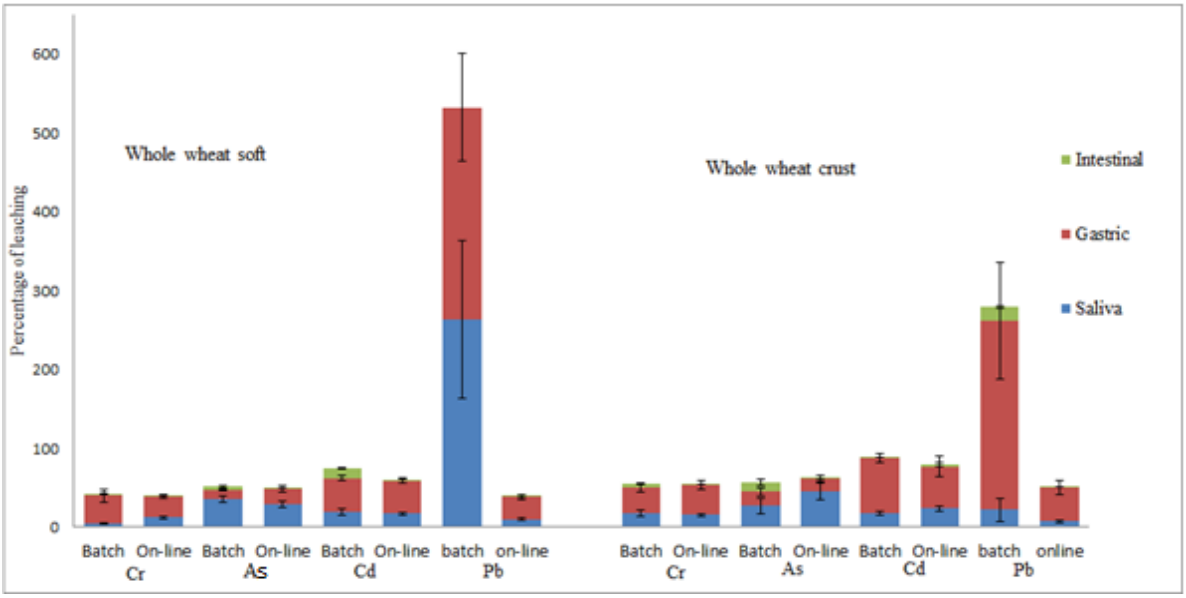

Figure 4.5 Bioaccessible fraction expressed as a percentage of the total concentration of Cr, As, Cd and Pb in soft and crust parts of whole wheat brown bread by batch and on-line methods

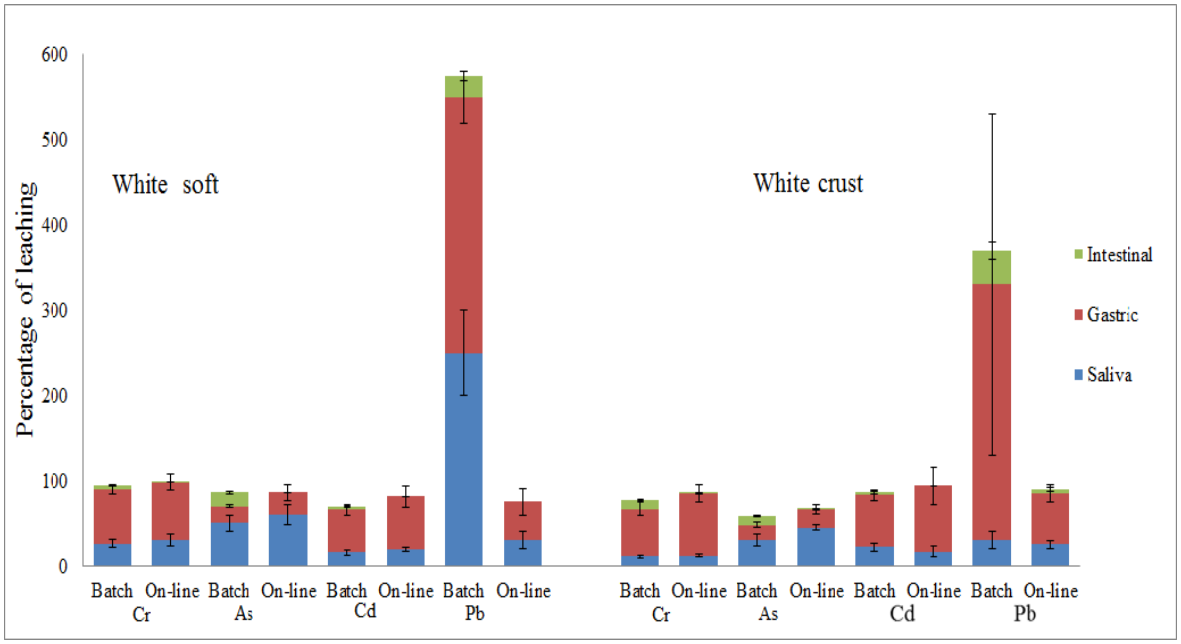

Figure 4.6. Bioaccessible fraction expressed as a percentage of the total concentration of Cr, As, Cd and Pb in soft and crust part of white bread by batch and on-line methods

A greater proportion of each element was released by intestinal juice when using the batch method than when using on-line leaching. These differences are likely due to the fact that the batch method determines equilibrium concentrations for analytes in a closed system whereas, with the on-line method, released analytes are continuously removed from the food substrate.

However, for both the on-line and batch methods, the total bioaccessible fraction was similar, according to a Student's t-test. Hence, the on-line leaching method gives similar results as those obtained with a batch method in addition to offering several benefits over the common batch method: it is more efficient, less susceptible to contamination, more dynamic, thereby providing real-time information, while being faster. It is thus best suited for accurate risk assessment. A contamination problem was experienced with the batch method in the case of Pb, a ubiquitous element, where mass balance could not be verified. Because on-line leaching drastically decreases sample manipulation, which only consists in loading the sample in a mini-column in contrast to batch methods requiring multiple centrifugation/decantation steps, contamination possibilities are minimized.

Figure 4.7 shows that all elements were more bioaccessible from white bread than whole wheat bread. This may be because of the larger number of steps involved in processing white bread than whole wheat bread, which may enhance bioaccessibility (Sensoy, 2013). Indeed, several food processing steps, such as bleaching and fortification of the flour with

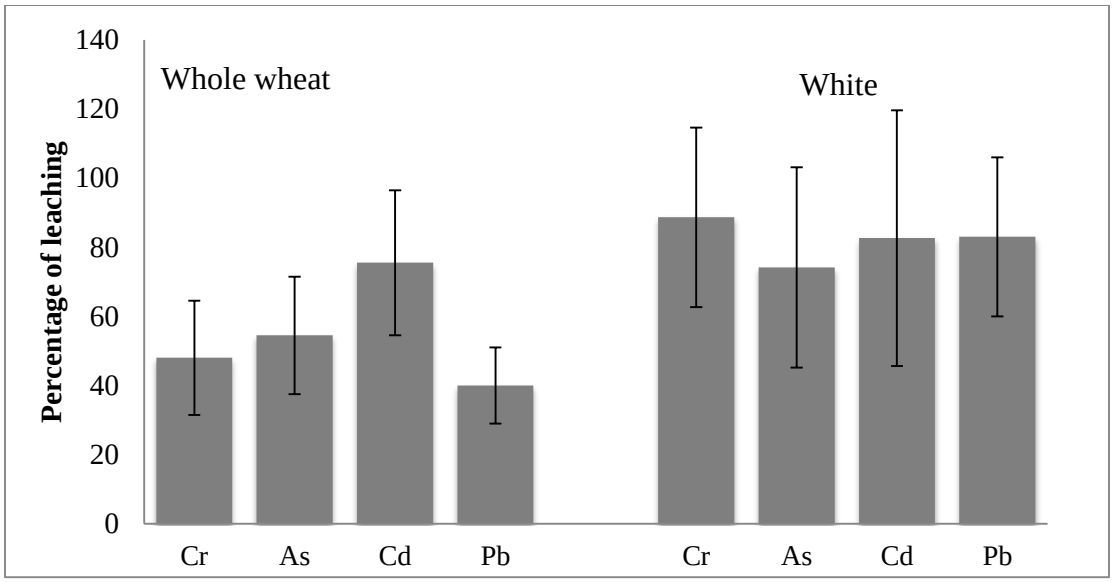

Figure 4.7 Average percentage (%) of total metal content present in the bioaccessible fraction of Cr, As and Cd by both batch and online leaching method and Pb by on-line leaching method in whole wheat and white bread.

the addition of essential nutrients, may cause a significant weakening in binding capability of the elements to the flour substrate. This facilitates the ease of food dissolution in the gastro-intestinal fluid. To the best of the author's knowledge, this is the first bioaccessibility study on the total toxic elements present in bread samples, simulating the human gastro-intestinal digestion system. Therefore, further research is needed to see if this difference applies to bread from other sources.

In fact, neither the batch nor the on-line leaching techniques are truly representative of the processes that actually occur in the human body. The gastro-intestinal tract is neither a stirred, closed system nor is it an open type system where metals are immediately absorbed as soon as they are released. However, the goal of this study was not to

accurately reproduce what happens in the body, as this is impossible to do, given the variety of digestion systems (for example, the HCl concentration in the gastric juice of vegetarians is lower than that of meat eaters).What is important for risk assessment is to have a method that assesses the worst case scenario, as if a food is safe under these conditions then it is safe for everybody.

The on-line approach provides a quick and simple means of assessing the bioaccessibility of elements and should therefore become an invaluable tool for the estimation of risks posed to human consumption. Government agencies, such as the Canadian Food Inspection Agency, can no longer rely on total element concentrations, as they provide no information on their bioaccessibility. The continuous on-line method coupled to ICP-MS is equally applicable to nutritional elements in food stuff.

4.3 Source of Pb in bread

Monitoring several isotopes of elements whose isotopic distribution varies in nature can help in further discriminating sources of this element. In the case of Pb, its $^{207}Pb/^{206}Pb$ $^{208}Pb/^{207}Pb$ isotope ratios were computed. For the batch method, these ratios were readily obtained by dividing the average steady-state signal for one isotope over that of the other. For the transient signal resulting from the on-line method, however, the point-by-point signal intensity of ^{207}Pb was plotted versus ^{206}Pb and that of ^{208}Pb versus ^{207}Pb in Microsoft Office Excel. This yielded a straight line whose slope was the corresponding isotope ratio (see the example in **Figure 4.7**). This approach was reported to provide

more accurate ratios than a ratio of peak areas or the average of point-by-point ratio (Epov *et al.* 2010). The resulting ratios are summarized in **Table 4.6**, where they are also compared to the normal Pb isotope ratios. As can be seen, the isotopic composition of Pb released by saliva was significantly different than that released by gastric juice in all cases, according to a Student's t-test at the 95% confidence level. This indicates that two different sources of Pb exist in whole wheat and white bread.

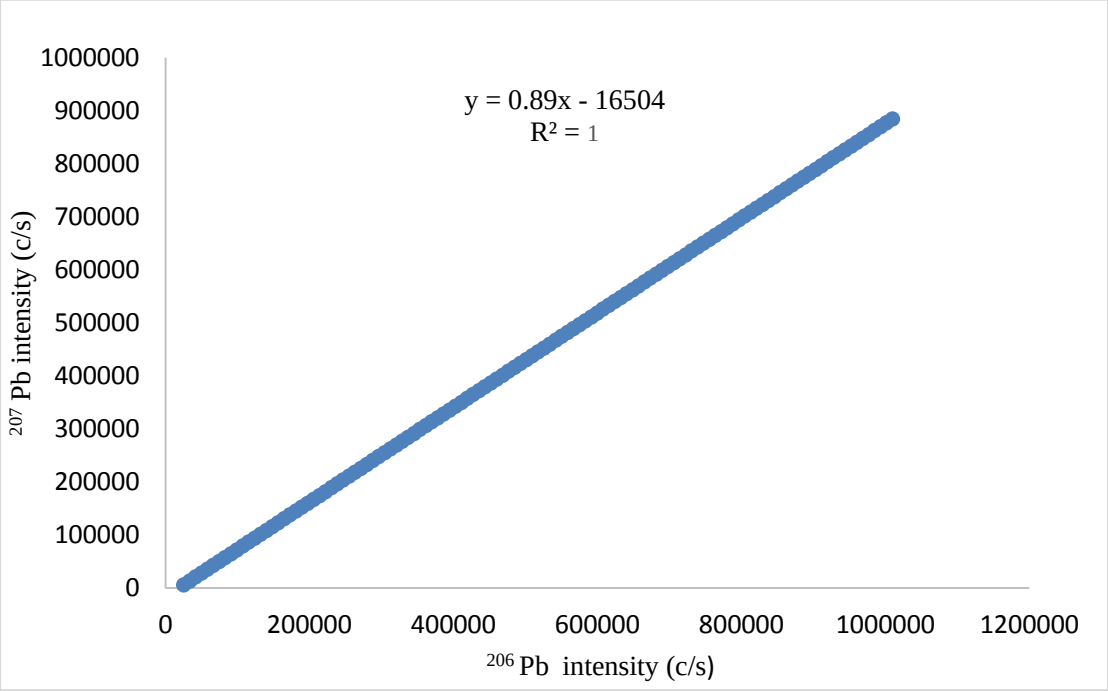

Figure 4.8 A typical plot of signal intensity of ^{207}Pb versus ^{206}Pb (n=71) observed with on-line continuous leaching of soft part of whole wheat bread with saliva.

Table 4.6 Isotope ratios (± standard deviation) measured for Pb in saliva and gastric juice by on-line continuous leaching and by the batch method (the number of points used to obtain the ratio is in parentheses**)** and Student's t-test comparison of the ratios measured in saliva and gastric juice at the 95% confidence level.

Sample	Method	Isotope	Reagent	Pb ratio		Student's t- test		Normal ratio
						Found	Table value	
Whole wheat soft	On-line	207/206	Saliva	0.89 ± 0.05	(n=71)	13.40	1.99	0.91
		207/206	Gastric	0.81 ± 0.01	(n=251)			
		208/207	Saliva	2.41 ± 0.02	(n=71)	16.28	1.99	2.37
		208/207	Gastric	2.37 ± 0.01	(n=251)			
White soft	On-line	207/206	Saliva	0.89 ± 0.03	(n=110)	16.32	1.98	0.91
		207/206	Gastric	0.84 ± 0.02	(n=335)			
		208/207	Saliva	2.43 ± 0.02	(n=110)	12.40	1.98	2.37
		208/207	Gastric	2.406 ± 0.006	(n=335)			
Whole wheat soft	Batch	207/206	Saliva	0.917 ± 0.004	(n=31)	44.76	2.05	0.91
		207/206	Gastric	0.846 ± 0.006	(n=18)			
		208/207	Saliva	2.40 ± 0.04	(n=31)	13.43	2.04	2.37
		208/207	Gastric	2.497 ± 0.003	(n=18)			
White soft	Batch	207/206	Saliva	0.80 ± 0.02	(n=18)	13.31	2.04	0.91
		207/206	Gastric	0.88 ± 0.03	(n=18)			
		208/207	Saliva	2.37 ± 0.05	(n=18)	10.91	2.04	2.37
		208/207	Gastric	2.52 ± 0.03	(n=18)			

4.4 Summary and perspective

In the previous discussion, we have optimized and employed the continuous on-line leaching method for ICP-MS to obtain the leaching profile of different toxic elements (Cr, Cd, As and Pb) in bread samples in order to measure the bioaccessible fraction for risk assessment. The on-line leaching method is an efficient, reliable and simple method that gives more similar results in a shorter time than conventional batch method.

The results reveal that the extraction of elements from bread begins in the oral cavity. As food proceeds to the stomach, more mobilization occurs due to a longer residence in a low pH environment. Very little leaching was observed in intestinal juice. For both the on-line and batch methods, the majority of elements were leached out by gastric juice, with the exception of As where the majority was released by saliva. While whole wheat bread contained a higher concentration of toxic elements than white bread, these elements were more bioaccessible from white bread than whole wheat bread.

The Food and Agricultural Organization (FAO)/World Health Organization established the provisional tolerable daily intake (PTDI) of Cr in bread as 3.33 μg kg^{-1} of body weight per day. Similarly, the PTDI for As, Cd and Pb are 2.1 μg kg^{-1}, 0.82 μg kg^{-1}, and 3.57 μg kg^{-1} body weight per day, respectively. Based on total concentration results of toxic elements in bread, an adult weighing 60 kg needs more than 18, 49, 12 and 18 bread slices (average weight of bread slice is 36-43 g) per day to reach the PTDI level for Cr,

As, Cd and Pb respectively. Similarly, a child at the age of 5 years (~ 18 kg), would need more than one and half slice of bread to reach PTDI levels for Cr and Pb, more than 4 slices for As and more than 1 bread slice to reach the PTDI levels. But based on the calculation of our bioaccessibility results, same adult requires more than 40, 90, 22 and 65 slices of bread per day to reach the PTDI levels for Cr, As, Cd, and Pb, respectively. Similarly, the same child would need 13, 27, 7 and 8 slices of bread per day to reach the PTDI levels for Cr, As, Cd, and Pb respectively. So, for a child, the consumption based on total concentration could be critical for Cr, Pb and Cd. However, based on bioaccessibility results, it seems completely safe for all ages. This suggests that bread consumption is within the safety limits for all populations and also emphasizes the essentiality in determining the bioaccessibility of elements from food sample.

Chapter 5

Conclusions and future work

In this project, the bioaccessibility of potentially toxic metals in bread was investigated, for the first time, by using a simple on-line continuous leaching method coupled to an ICP-MS instrument equipped with a CRI. This on-line approach demonstrated similar results to those obtained by using a conventional batch method. Mass balance was verified for both methods and confirmed by statistical evaluation (at a 95% confidence level). Hydrogen CRI gas was efficient in minimizing carbon- and chlorine-based polyatomic interferences.

Compared with conventional batch methods, the on-line leaching method efficiently provides more representative leaching data on the bioaccessibility of toxic elements in bread. It has several advantages over conventional methods such as small sample consumption, minimal sample manipulation, ease of execution, real time process monitoring and simple data interpretation. Furthermore, this method is less susceptible to contamination during sample preparation, which is performed in a closed system. Thus it is more suitable for risk assessment. While this was not observed in this work, it also allows the distinction of the dissolution of different phases with different reagents (Beauchemin, Chipley & Kyser, 2002). The use of ICP-MS also enables a continuous monitoring of isotope compositions, which in turn allows the identification of different sources of elements (whose distribution varies in nature).

In the future, the on-line leaching method will be applied to a broader range of bread samples for the analysis of the bioaccessible toxic elements. It has been shown that the total concentration of elements or compounds in food products does not necessarily predict the nutritional or toxicological information of the food products. In reality, the toxic/nutritional properties of elements depend significantly on their chemical form. For instance, chromium exists mainly in two different oxidation states, namely Cr (III) and Cr (VI). The behavior of the two species is significantly different in biological and toxicological contexts (Xing & Beauchemin, 2010). Cr (III) is an essential element in the human body and plays an important role in the metabolism of glucose, protein and lipids. Cr (VI) on the other hand, is detrimental to health, even at relatively low levels; it may be involved in the pathogenesis of some diseases such as liver, kidney, lung and gastrointestinal cancers. Therefore, further work will be focused on the speciation analysis of the bioaccessible fraction of elements in bread samples using ICP-MS coupled with ion exchange chromatography.

Work will also focus on the bioaccessibility studies of the different elements in toasted bread samples to see the effect of heating on bioaccessibility. As well, unlike in this work, saliva containing enzyme will be used to see if the enzyme has a significant effect on bioaccessibility. Indeed, the most important factor affecting the release of elements appears to be the chemical environment of the gastro-intestinal tract. Further studies will aim to better understand the effect of other factors, such as the leaching flow rate, nature of leaching reagents, heating or cooking of the food, and the dimensions of the mini-

column used so as to improve the reproducibility of the continuous on-line leaching method.

In this work, a relatively large relative standard deviation was observed for replicate analyses by both methods for the selected elements in bread slice. So, future work will also study of the distribution of elements in a bread slice by laser ablation coupled to ICP-MS.

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