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Chromium redox speciation in food samples

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Abstract: Chromium is an element with important biological characteristics, depending on its different species. Cr(III) is considered as essential; however, the Cr(VI) form is classified as carcinogenic. For this reason, speciation analysis in food samples is a very important question. The proposed data procedures for chromium redox speciation in the literature can be divided into those with the use of online hyphenated techniques and those where appropriate sample pretreatment is necessary. The strategies for nonchromatographic speciation are mainly based on selective liquid–liquid extraction procedures, coprecipitation, selective separation of chromium species using a solid phase extraction column, or complexation reactions. In this paper, the application of these strategies for determination of Cr(III) and Cr(VI) contents in common food samples (water, milk, tea infusion, bread, and beer) are presented and discussed. This survey is an attempt to cover the state of the art since 2012.

Key words: Chromium, speciation analysis, food samples

1. Introduction

Chromium is extensively used in industrial processes (electroplating, metal finishing, leather tanning, production of pigments). Chromium compounds are discharged into air, water, and terrestrial environments in their most stable oxidation states as Cr(III) and Cr(VI). The biological and toxicological properties of these two redox forms are significantly different. Cr(III) is considered as an essential element for the proper functioning of living organisms, particularly in glucose metabolism.[1] Thus, it is often added to vitamins in dietary supplements. Cr(VI) with its high oxidation potential shows mutagenic and carcinogenic effects and is regarded as carcinogenic by the World Health Organization.[2] The maximum concentrations of total chromium and Cr(VI) in drinking water were set as 50 μg/L and 20 μg/L, respectively, according to European Council Directive 98/83/EC.[3] Chromium is not considered essential to plants and is mainly accumulated in roots, independent of its species.[4] However, its presence affects photosynthesis, germination, growth, and yield.[5] Some plants that can accumulate a great amount of this metal may be used for its removal from the soil.[4]

According to IUPAC guidelines, speciation analysis is defined as the analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a given sample.[6] This procedure usually involves initial separation of species, followed by their determination. When analyzing solid samples, an analytical scheme also involves isolation of the appropriate species, mainly by extraction. The separation of species is the most critical step in the analytical speciation procedure as species may be converted from one form to another, or even lost from a sample. It is necessary to quantitatively extract chromium species from the

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complex biological matrix without interconversion between species. Cr(III) is stable at lower pH and Eh values, while higher pH and Eh values stabilize the presence of Cr(VI).[7] Thus, when acidic extraction conditions are applied, Cr(VI) could be converted to Cr(III).

There is a group of analytical techniques, mostly X-ray-based spectroscopy, which enables solid-state speciation analysis directly in the sample. X-ray photoelectron spectroscopy (XPS) and X-ray absorption near-edge spectroscopy (XANES) are less prone to speciation conversion but they have higher detection limits in comparison to wet-chemistry analytical techniques.[8] However, these methods have not yet been applied for chromium speciation in solid food samples; they have only been used for contaminated soils, sediments, and coatings.[9]

Analytical procedures proposed for chromium redox speciation can be divided into those with the use of online hyphenated techniques and those where appropriate pretreatment of the sample is necessary (nonchromatographic speciation). The strategies for nonchromatographic speciation are based on selective liquid–liquid extraction procedures, coprecipitation, selective separation of one or two chromium species onto a solid phase extraction column, or complexation reactions. Such approaches also enable the preconcentration of given species. The content of the second species is then calculated by the difference after determination of total chromium preceded by reduction of Cr(VI) or oxidation of Cr(III).

Several reviews focused on speciation analysis of chromium.[8,10–13] They were mostly focused on determination of both Cr species in environmental samples such as natural water, sediments, waste, or soils. Analytical strategies for separation and preconcentration techniques[10,12,13] as well as progress in miniaturization of measurement systems based on different extraction techniques were also critically discussed.[10,13] The paper by Zhou et al.[11] dealt with preconcentration procedures for the determination of chromium following only atomic spectrometric techniques. The different directives and recommendations for chromium speciation in solid matrices (soils, sledges, sediments, and industrially produced samples) were reviewed by Uceta et al.[8]

In spite of the great number of papers about chromium speciation in different kinds of water, there is considerably less information regarding its species in food samples, e.g., plants, vegetables, or grains and the final products obtained from them, which are consumed by humans and animals. The increasing pollution of all environmental compartments can contribute to the contamination of plants with some pollutants, including chromium compounds. These chromium forms differ highly with respect to their chemical properties and biological activities. In the acidic environment of the stomach, ingestion of the more toxic Cr(VI) should be reduced. However, some published papers reported considerable amounts of Cr(VI) in the edible parts of plants. The interest in Cr(VI) determination in food samples still remains under discussion.[14,15] The present paper is focused mainly on the application of different analytical strategies for determination of Cr(III) and Cr(VI) contents in common food samples such as water, milk, bread, tea infusion, beer, and dietary supplements. This survey is an attempt to cover the state of the art since 2012. Earlier developments were presented in the mentioned previous reviews and since that time several new procedures for chromium speciation have been developed.

2. Hyphenated techniques

High-performance liquid chromatography (HPLC) is the most commonly applied technique for separation of chromium species,[16,17] whereas capillary electrophoresis[18] is used to a lesser extent. Ion-pair reversed-phase chromatography (IPC-RP)[19–21] and ion chromatography (IC)[22,23] are the main modes for separation. When an

anion-exchange column is used, Cr(III) species should be converted into a negatively charged complex, usually with EDTA. This derivatization step also prevents hydrolysis of Cr(III) in the neutral pH range. An elevated temperature (up to 70 °C) was usually applied to accelerate the complexation reaction. Isocratic elution with a solution containing NH_4NO_3, Na_2EDTA, or diluted NaOH was used for separation of the $[CrEDTA]^-$ complex from Cr(VI). In RP mode, tetraethylammonium or tetrabutylammonium salts were employed as ion-pair reagents together with EDTA.

The coupling of HPLC with inductively coupled plasma mass spectrometry (ICP-MS) is now the most common technique for determination of Cr speciation due to its high sensitivity, the wide linear concentration range, and multiple-isotope detection.[16] Isotope ^{52}Cr is preferred due to its great abundance (83.8%). Quantification is usually affected by polyatomic species ($^{40}Ar^{12}C^+$, $^{40}Ar^{13}C^+$, $^{35}Cl^{16}OH^+$, $^{37}Cl^{16}O^+$), which are formed between gaseous plasma, reagents, and the sample matrix. The application of collision or reaction cell, cool plasma conditions, high-resolution mass spectrometer, and mathematical correction as well as the optimization of the chromatographic parameters for separation allow for the removal of these interferences.[16,17] The limits of detection (LODs) for determining both chromium species vary in the range of 0.02–0.10 μg/L.[19–23] To control interconversion of chromium species during the analytical procedure, speciated isotope dilution ICP-MS was proposed.[16,17]

3. Nonchromatographic separation

The nonchromatographic speciation analysis of chromium still receives increasing interest since the content of its different redox forms in natural samples is very low and a preconcentration step is necessary to enrich the analytes. The most widely used techniques for this purpose include solvent extraction carried out at a reduced scale such as dispersive liquid–liquid microextraction (DLLME), cloud point extraction (CPE), and solidified floating organic drop microextraction (SFODME), as well as solid phase extraction (SPE) with appropriate solid materials. These techniques permit the obtaining of a high enrichment factor, easy and fast phase separation, and the automation of sample pretreatment.

In DLLME, for extraction of Cr(VI), tributylphospate (dispersion was achieved by ultrasounds)[24] and ammonium pyrrolidine-dithiocarbamate (APDC) with CCl_4 were used as disperser solvents.[25] Room temperature ionic liquids were proposed as a new green solvent.[26,27] The highest efficiency of Cr(VI) extraction using 1-hexyl-3-methylimidazolium hexafluorophosphate ([Hmim][PF_6]) was obtained with the pH range of 1–3, while extraction of Cr(III) was significant only at pH 5.[27] The LOD of the proposed method for tap and mineral water samples was 3 μg/L (detection with ICP-OES).

Recently, the combination of dispersive liquid–liquid microextraction with SFODME has been proposed for speciation analysis of chromium in water.[28–30] The cationic complex of Cr(VI) with 1,5-diphenylcarbazide (DPC) was extracted with the coacervative phase as an ion pair with sodium dodecyl sulfate (SDS) using decanoic acid and dispersed in tetrahydrofuran–water mixtures.[28] Tetrahydrofuran plays a double role as a dispersing solvent and also in the self-assembly of decanoic acid. The extraction phase was solidified on an ice bath and after transfer into a conical vial, it melted immediately at room temperature. It was then diluted with acetonitrile and transferred to a microcell, where absorbance of the complex was measured at 540 nm. The LOD using spectrophotometric detection was 0.23 μg/L,[28] while with ETAAS detection it was 0.003 μg/L.[29]

Meeravai et al. proposed sequential or simultaneous solidified floating organic drop microextraction of chromium species.[30] In the sequential approach, Cr(III) was extracted with 1-undodecanol in the presence

of the anionic surfactant SDS. Cr(VI), converted into a cationic Cr(III)-DPC complex, was extracted using a similar procedure. In the simultaneous procedure, both Cr species were extracted using the above procedure in the presence of diphenylcarbazone to determine the total chromium content. The detection limits for Cr(III) and Cr(VI) in sequence and total Cr in simultaneous modes were 3.1 and 4 pg/L, respectively.

Using the cloud point extraction procedure, chromium species were transferred to the surfactant phase of Triton X-114 in the presence of silver nanoparticles (AgNPs).[31] AgNPs contained an excess of reductant ($NaHB_4$), which was used for their preparation; thus, Cr(VI) was reduced and interacted with the nanoparticles together with Cr(III). The analytical signal after injection of the surfactant-rich phase to ETAAS was due to total chromium content.[31] For speciation study, the procedure was repeated by addition of EDTA solution immediately before mixing with the AgNP suspension and the obtained signal corresponded to Cr(VI). The extreme sensitivity of the proposed procedure was reported with a LOD of 2 ng/L and an enrichment factor of 1150. It was possible to measure the content of both chromium species in diluted water, wine, and beer samples.

Solid phase extraction coupled with different detection systems is the most popular separation technique for speciation analysis of chromium due to its advantages, such as high enrichment factor, absence of emulsion, ability to be applied to field sampling, and easy automation using online approaches.[10,13] The current research in SPE is focused on the development of new sorbents based on nanostructured materials, magnetic nanoparticles, ion-imprinted polymers, and mesoporous silica.[32,33] Whereas monofunctional nanomaterials provide a single function, hybrid nanoparticles combine the properties of their nanoconstituents, which can be highly useful in simplifying analytical methods as well as exploring new challenges and applications relying on their synergistic effects.[34] Such hybrid nanomaterials were applied for chromium redox speciation.[35–39] The homogeneous distribution of dispersed nanoparticles, such as Fe_3O_4, in solution causes their easy separation with the aid of an external magnetic field.

Dispersive SPE considerably reduces the time and simplifies the extraction process. It is not carried out in a column, cartridge, or disk, but a portion of sorbent is dispersed in the liquid sample.[35–38,40] Compared with classic SPE methods, preconditioning of the sorbent is not necessary, simplifying its performance and reducing the extraction time.

Recent developments using the SPE technique for redox speciation of chromium are presented in Table 1. The proposed procedures for redox speciation analysis of chromium include selective retention of one or both chromium redox species in a single or dual column system, followed by selective elution. The content of the second form is then determined after its reduction or oxidation and calculated as the difference between determined total chromium and initially determined Cr form.

4. Chromium species in food samples

Chromium absorption is relatively low ($<10\%$ of the ingested dose). It has been suggested that most of the ingested Cr(VI) is reduced in the gastrointestinal tract under acidic conditions.[14] Thus, Cr(VI) seems to be absent in food and its presence in drinking water is usually a consequence of anthropogenic activity. There are currently no maximum levels in the European Union legislation for chromium, whether Cr(III), Cr(VI), or total, in foodstuffs, except water. The maximum limit of 50 μg/L for total chromium in natural mineral water was laid down in Commission Directive 2003/40/EC.[57]

The extraction of Cr species from solid food samples is one of the most critical steps in the whole analytical procedure. The main difficulty is to preserve the initial distribution of both redox chromium species

Table 1. Recent developments for redox speciation of chromium using solid phase extraction.

Sorbent	Sorption conditions		Eluent	Enrichment factor		Detection (LOD, μg/L)		Ref.
	Cr(III)	Cr(VI)		Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	
Amino-functionalized Fe_3O_4/SiO_2	-	pH 5	HCl	-	16		FAAS (1.1)	35
Fe_3O_4@ZrO_2 nanoparticles	pH 8–9	-	HNO_3	25	-	FAAS (0.69)	-	36
Fe_3O_4@polyaniline	-	pH 7.6		-		-	UV-Vis (0.1)	37
Fe_3O_4@SiO_2 modified with chitosan	pH 9		HNO_3	100		ICP-OES (0.02)		38
Reduced graphene with SiO_2	-	DPC[1] + SDS	EtOH	-	167	-	UV-VIS (0.4)	39
Chitosan film with Ag nanoparticles	pH 8.5	-	HNO_3			-	ICP-MS (0.02)	40
MWCNTs[2] modified with Aliquat 336	-	pH 2	HNO_3				TXRF[3] (3)	41
MAD[4]	pH 3	-	HCl	200	-	FAAS (0.01)	-	42
MAD[4] for Cr(III) Dowex 21K for Cr(VI)	pH 3	pH 3	HNO_3	48	30	FAAS (0.05)	FAAS (0.3)	43
Amberlite IRA-910 for Cr(VI) DPTH-gel[5] for Cr(III)	pH 5.5	pH 5.5	HNO_3	2.4	3.7	ICP-MS (0.03)	ICP-MS (0.1)	44
Functional polymer[6]	EDTA + TBAH[7]		TBAH[4]	105	128	HPLC-ICP-MS (0.0068)	HPLC-ICP-MS (0.0041)	45
Poly(methacrylic acid) for Cr(III) Polyvinylimidazole for Cr(VI)	pH 4	pH 4	HNO_3	47.3	8.6	FAAS (0.84)	FAAS (1.58)	46
Mesoporous silica NH_2-SBA-15	pH 2	-	NH_3	44	-	FAAS (0.2)	-	47
$SiO_2/Al_2O_3/TiO_2$ for Cr(III) SiO_2/AAPTS[8] for Cr(VI)	pH 5	pH 5	HCl	17.6	33	FAAS (0.66)	FAAS (0.27)	48
Lysine-SBA-15	-	pH 5	NH_3	-	91	-	SCGA-AES[9] (0.75)	49
Polyoxometalate cluster	pH 3	-	HNO_3		-	ICP-MS (0.5)	ICP-MS (0.3)	50
TiO_2 nanotubes	pH 5–8	-	HNO_3	67	-	ICP-MS (0.0075)		51
Ni-Al LDH[10]	-	pH 6	NH_2OH	-	100	-	FAAS (0.510)	52
Moringa oleifera husks	pH 7–9	pH 1–2	HNO_3	2.5		FAAS (1.92)	FAAS (2.45)	53
MWCNTs[8] modified with AAPTS[5]	-	pH 2.2	HNO_3	-	10	-	ICP-MS (0.016)	54
Imprinted polymer with 8-hydroxyquinoline	pH 9	-	HNO_3	33	-	FAAS (2.1)	-	55
Imprinted polymer with APDC[11]	pH 3.5–4.8	-	HNO_3	10	-	ETAAS (0.018)	-	56

[1] Dipehylcarbazide; [2] multiwalled carbon nanotube; [3] total reflection X-ray fluorescence; [4] poly-2-(5-methylylisoxazole) methacrylamide-co-2-acrylamido-2-methyl-1-propanesulfonic acid-co-divinylbenzene; [5] silica gel functionalized with 1-(di-2-pyridyl)methylene thiocarbonohydrazide; [6] N,N-bis(2-aminoethyl)ethane-1,2-diamine functionalized poly(chloromethyl styrene-co-styrene); [7] tetrabutylammonium hydroxide; [8] silica gel functionalized with [3-(2-aminoethylamino)propyl] trimethoxysilane; [9] solution-cathode glow discharge-atomic emission spectrometry; [10] nickel-aluminum layered double hydroxide; [11] pyrrolidinedithiocarbamate.

in the sample as well as to obtain high extraction efficiency.[8] The analytical procedure for chromium speciation in food depends on the nature of the sample matrix. Beverages such as water, beer, or tea infusions do not need an extraction step. Thus, the procedure does not include the additional step. However, pH, exposure to light, temperature, the type of storage container, and high storage temperatures may affect the stability of chromium species.[10,11]

4.1. Water

Cr(VI) appears to be present in tap water at levels in the range from 0.055 μg/L [30] up to 13.36 μg/L[47] (Table 2). In only one work [58], its concentration was below the LOD of SPE-FAAS procedures (0.51 μg/L). The presence of Cr(VI) in drinking water could be the consequence of anthropogenic contamination. As water treatment facilities use strong oxidants, chromium may easily be present in the hexavalent state. López-Garcia et al. did not find Cr(III) in tap water samples (LOD was 2 ng/L), but Cr(VI) was present at a concentration level of 0.095 μg/L.[31]

Table 2. Concentration of chromium species (in μg/L) in tap and mineral water using different procedures.

	Cr(III)	Cr(VI)	Procedure	Ref.
Tap water	0.080 ± 0.015	0.160 ± 0.007	HPLC-ICP-MS	21
	0.57 ± 0.025	0.320 ± 0.019	SFODME-GFAAS	57
	n.d.	0.095 ± 0.005	DLLME-GFAAS	31
	5.5 ± 0.3	1.7 ± 0.3	DLLME-SFODME-Vis	28
	0.312 ± 0.016	0.055 ± 0.023	CPE-GFAAS	30
	4.41 ± 0.65	13.36 ± 0.70	SPE-FAAS	47
	5.2 ± 0.7	n.d.	SPE-FAAS	51
Mineral water	0.682 ± 0.028	n.d.	SFODME-GFAAS	58
	0.012 ± 0.002	0.027 ± 0.003	DLLME-GFAAS	31
	2.42 ± 0.25	n.d.	SPE-FAAS	47

SFODME – Solidified floating organic drop microextraction, DLLME – dispersive liquid–liquid microextraction, CPE – cloud point extraction; SPE – solid phase extraction.

The concentrations of chromium species in mineral waters were quantified far less often (Table 2). Cr(VI) was found only in one sample at a level of 0.027 μg/L.[31] In the same water sample, the determined concentration of Cr(III) was lower (0.012 μg/L).

4.2. Beer and wine

Viera et al. conducted the study of the presence of total and hexavalent chromium in different styles of lager beers packaged in glass or cans.[58] For selective extraction of Cr(VI), the SPE procedure utilizing Chromabond NH_2 was applied. A similar procedure was earlier applied by the same group for milk matrices.[59,60] The detection limit for Cr was 1.61 μg/L using GFAA detection.[58] The concentration of total Cr in bottled beer was up to 16.4 μg/L, while in canned beer it was up to 2.79 μg/L. From among 70 analyzed beers, only in 5 samples was the content of Cr(VI) higher than the LOD of the proposed procedure (in the range of 2.52–13.0 μg/L). These samples also contained higher level of total chromium.[58]

Cr(VI) was found in red and white wine samples in the range of 61–135 ng/L and 51–65 ng/L, respectively.[31] The presence of ethanol affected the enrichment/separation step using cloud point extraction

of silver nanoparticles by Triton X-114; thus, the samples were diluted four times before the preconcentration procedure.

4.3. Tea leaves and infusions

The extraction of Cr(VI) from tea leaves and other plant samples was done by leaching with sodium carbonate at elevated temperatures.[61–63] This alkaline digestion procedure was recommended for extraction of Cr(VI) from adsorbed and precipitated chromium compounds in soils, sediments, and sludges as the US EPA 3060A method.

Under these conditions, Cr(III) species form insoluble hydroxides or carbonates. The validation of the proposed procedure was done by spiking tea leaves with Cr(VI) standard solutions just before treatment with Na_2CO_3 and good recovery (98 ± 5%) was obtained. From the obtained results, the conclusion was made that Cr(VI) was not altered during extraction process.[61–63] However, in those studies, electrothermal AAS was applied for quantification of chromium content in the leaching solutions. By this method only total Cr is measured, although it was stated that the determined concentrations were for Cr(VI).

According to Mandiwana et al.[63] higher content of Cr(VI) was found in black tea (in the range of 0.03–3.15 μg/g) than in green tea (0.03–0.14 μg/g) or herbal tea (below the LOD of 0.020 μg/g). Taking into account these data, up to 17.5 μg/L of Cr(VI) could be consumed in a typical cup of black tea (2.0 g of tea leaves or standard teabag extracted using 200 mL of hot water). Although this is below the maximum acceptable concentration (50 μg/L) of total chromium in beverages[56], the California Department of Public Health announced in 2014 a regulation establishing the drinking water standard for Cr(VI) of 10 μg/L.[64] As tea is one of the most commonly consumed beverages worldwide, with every cup up to 3.5 μg of this Cr redox species can be consumed, and many people drink more than one cup of tea daily. Thus, consumption of this common drink could represent long-term chronic exposure to Cr(VI) with health hazards.

Tea leaves and the infusions prepared from them contain several flavonoids with strong antioxidant activity and they are primarily responsible for the beneficial healthful properties of tea.[65] The presence of a reducing organic matrix in tea samples inhibits and prevents the existence of Cr(VI) species. The analytical application of this redox reaction between quercetin (one of the most abundant flavonoids present in plants) and Cr(VI) was utilized for determination of chromium species in water samples.[66]

Novotnik et al.[14] also used alkaline extraction for leaching Cr species from different tea leaves and applied speciation analysis by HPLC-ICP-MS. ^{53}Cr(III) and ^{50}Cr(VI) stable isotopes were used to check the interconversion of chromium species during the extraction step. Figure 1 shows the chromatograms of the alkaline extract of tea leaves (Figure 1A) and tea infusion (Figure 1B) recorded after they were doubly spiked with stable isotopes of Cr(III) and Cr(VI).[14] A Cr(VI) peak was not detected at m/z 52 (t_R 430–470 s) in either sample. The addition of ^{50}Cr(VI) to the extract caused its reduction but the added ^{53}Cr(III) was not oxidized. Thus, it was proved that Cr(VI) cannot exist in tea infusion in the presence of such an organic matrix containing antioxidants. Similar results were obtained for several beverages (wine, fruit juices, tea) using alkaline extraction (aqueous NH_3 solution at pH 11.5) followed by determination of Cr(VI) by HPLC-ICP-MS.[23] The speciation analysis data also confirmed that when Cr(VI) was added to the aqueous extract of Neem powder used traditionally in Ayurvedic medicine in India[67] or to edible animal oil[68], it was rapidly reduced by the presence of antioxidants.

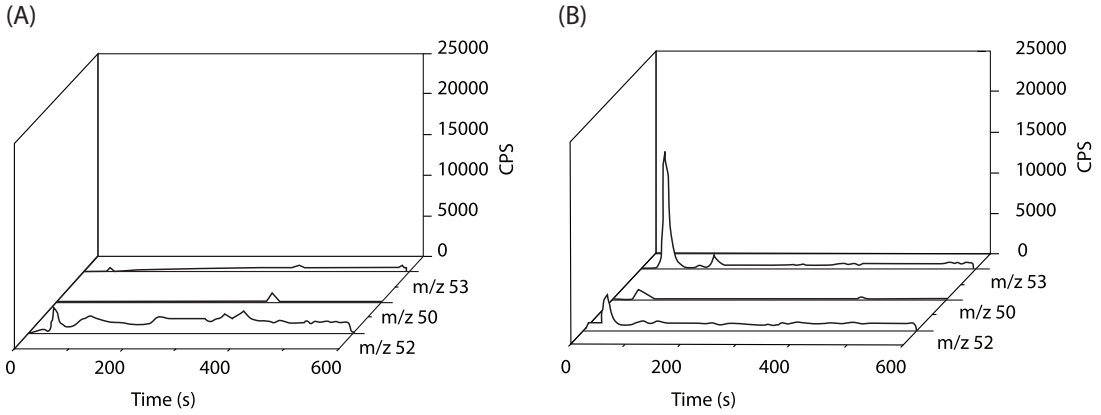

Figure 1. Chromatograms of (A) tea leaves and (B) tea infusion alkaline extract (0.1 mol/L Na_2CO_3) obtained by an HPLC-ICP-MS procedure, recorded at m/z 50, 52, and 53. Samples were double-spiked with 10 μg/L of ^{50}Cr(VI) and 10 μg/L of ^{53}Cr(III). Reproduced from Ref. 14 with permission from the Royal Society of Chemistry.

Chen et al.[51] proposed speciation analysis in tea infusions by separation of positively charged Cr(III) species on the surface of negatively charged TiO_2 nanotubes. The fraction that was not adsorbed was described as Cr(VI) and determined by ETAAS. The authors did not consider the composition of the matrix with several organic ligands, which can form negatively charged or neutral complexes with Cr(III) and not be retained on TiO_2 nanotube surfaces. Thus, the reports[58,61–63] concerning the content of Cr(VI) in some foodstuffs with ETAAS detection without its identification are artifacts of inappropriately applied analytical methodology and may lead to mistaken interpretations.

4.4. Milk and dairy products

The redox speciation of chromium in milk and dairy products is very important due to their nutritional values. Ambushe et al.[69] determined the concentration of total chromium and Cr(VI) in several different brands of pasteurized cow's milk purchased from supermarkets in Tshwane, South Africa. Cr(VI) was selectively adsorbed on Chromabond NH_2, the ion-exchange column, and after subsequent elution by nitric acid solution determined by ICP MS using dynamic reaction cell with O_2 as a reactive gas to minimize interferences from polyatomic ions. The LODs of this procedure were 0.091 and 0.085 μg/L for total Cr and Cr(VI), respectively. For total Cr and Cr(VI) levels in the ranges of 33.2–57.1 μg/L and 0.61–1.44 μg/L were detected, respectively.

Alkaline extraction followed by Cr(VI) determination using HPLC-ICP-MS was applied for several milk samples (cow, soy, goat, whole, and powder) as well as for yogurt and different cheeses.[23] The samples were first centrifuged and the upper layer of fat was discarded. Then the samples were diluted with NH_4OH solution (pH 11.5) and put into an ultrasonic bath for 1 h to release Cr(VI) from the matrix. The obtained solution was ultrafiltered (cutoff: 10 kDa) for protein removal and analyzed. For dairy products the LOD was 1 μg/L. Cr(VI) was not determined in any of the analyzed samples. In order to check the possibility of interconversion of chromium species, stability studies were conducted. The rate of Cr(VI) reduction was dependent on the temperature as the loss of this form was more than ten times faster at room temperature than at –18 °C. In other food samples, such as rice, different fruits and vegetables, wine, chocolate, and meat, the content of Cr(VI) was also below the LOD.[23]

4.5. Flour and bread

Products made with wheat and other cereals are among the major constituents of the human diet. For this reason, the determination of the toxic Cr(VI) form is very important. Commercial bread samples (n = 152) were collected from a local market in Porto, Portugal, and Cr(VI) was extracted from these samples using a 0.01 M NaOH solution over 17 h at room temperature.[70] After centrifugation, its content was determined in supernatant by ETAAS method. The limit of quantification of the proposed analytical procedure was 4.95 and 5.60 μg/kg for total chromium and Cr(VI), respectively. The mean values for total chromium and Cr(VI) in white bread and wholegrain bread samples were 5.65 $\pm$ 5.44 and 6.83 $\pm$ 4.88 μg/kg of dry weight, respectively. It was slightly above 10% of the total chromium content. Based on these results, the mean daily intake of Cr(VI) was estimated (considering three bread units with a weight of about 50 g) as 0.57 and 0.69 μg/kg for white and wholegrain bread, respectively.

The experiments regarding the presence of Cr(VI) in bread samples were repeated by Novotnik et al.[14] The speciation procedure in alkaline extracts, doubly spiked with enriched stable isotopes of both Cr redox forms, was carried out by HPLC-ICP-MS to check the possibility of species interconversion during extraction. The chromatograms of these experiments are presented in Figures 2A and 2B. The added ^{53}Cr(III) was not oxidized and ^{53}Cr(VI) was partially reduced in the white bread extracts ($\sim$20%) and in the wholegrain bread extract ($\sim$60%). Thus, the organic matrix present in bread could reduce Cr(VI) in highly alkaline conditions. The speciation analysis performed by HPLC-ICP-MS of alkaline extracts of several flour and bread samples also confirmed that the Cr(VI) content was below the limit of detection (10 μg/kg).[23]

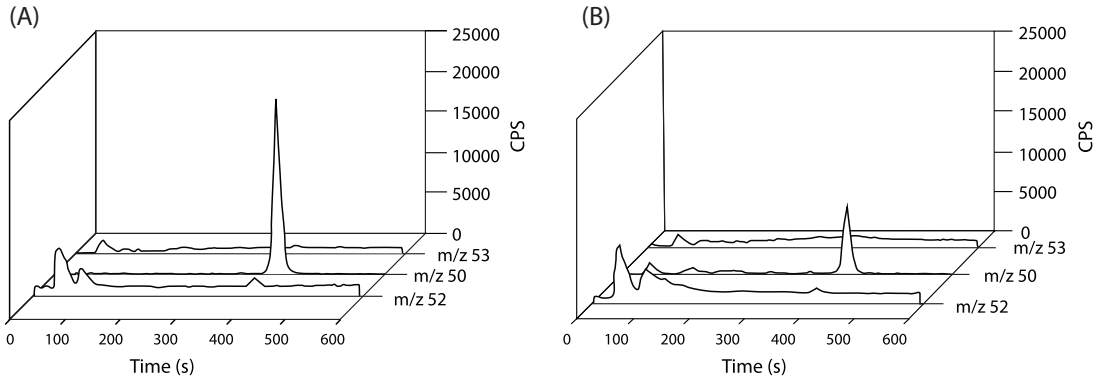

Figure 2. Chromatograms of (A) white bread and (B) wholegrain bread alkaline extract (0.1 mol/L Na_2CO_3) obtained by an HPLC-ICP-MS procedure, recorded at m/z 50, 52, and 53. Samples were double-spiked with 10 μg/L of ^{50}Cr(VI) and 10 μg/L of ^{53}Cr(III). Reproduced from Ref. 14 with permission from the Royal Society of Chemistry.

5. Conclusions

Chromium is one of the most often cited metals when it comes to discussing the necessity of speciation analysis. Its two stable oxidation states, Cr(III) and Cr(VI), highly differ with respect to chemical properties and biological activities. The determination of the Cr(VI) form, classified as carcinogenic in food of plant or animal origin, is particularly important.

Very low chromium content, especially Cr(VI), in such samples requires the use of sensitive and specific analytical methods. There is also the possibility of interconversion of Cr species due to the presence of an organic matrix containing reducing or oxidizing agents. The online coupling of HPLC with ICP-MS detection

seems to be the best solution for this purpose. The application of the reaction cell could reduce polyatomic interferences. Moreover, by use of enriched stable isotopes of ^{53}Cr(III) and ^{50}Cr(VI), it is possible to check the interconversion of chromium species during the whole analytical procedure. The proposed analytical procedures that are applied not specifically for Cr(VI) quantification methods (such as ETAAS) produce erroneous results regarding the presence of this chromium form in common food samples.

References

1. Pechova, A.; Pavlata, L. *Vet. Med.* **2007**, *52*, 1-18.
2. World Health Organization. *Chromium in Drinking-Water: Background Document for Development of WHO Guidelines for Drinking-Water Quality*; Word Health Organization: Geneva, Switzerland, 2003.
3. European Council. *Council Directive 98/83/EC of November 1998 on the Quality of Water Intended for Human Consumption*; European Council: Brussels, Belgium, 1998.
4. Oliveira, H. *Journal of Botany* **2012**, *2012*, 375843.
5. Kováčik, J.; Klejdus, B.; Babula, P.; Soares, M. E.; Hedbavny, J.; de Lourdes Bastos, M. *J. Inorg. Biochem.* **2015**, *145*, 70-82.
6. Templeton, D. M.; Ariese, F.; Cornelis, R.; Danielsson L. G.; Muntau, H.; van Leeuwen, H. P.; Łobiński, R. *Pure Appl. Chem.* **2000**, *72*, 1453-1470.
7. Mizanur Rahman, R. M.; Pamuku, M.; Kingston, H. M. *J. Agric. Food Chem.* **2013**, *61*, 9966-9976.
8. Unceta, N.; Séby, F.; Malherbe, J.; Donard, O. F. X. *Anal. Bioanal. Chem.* **2010**, *397*, 1097-1111.
9. Malherbe, J.; Claverie, F. *Anal. Chim. Acta* **2013**, *773*, 37-44.
10. Pyrzynska, K. *TrAC-Trend. Anal. Chem.* **2012**, *32*, 100-112.
11. Zhao, X.; Song, N.; Zhou, W.; Jia, Q. *Cent. Eur. J. Chem.* **2012**, *10*, 927-937.
12. Pyrzynska, K. *Int. J. Environ. An. Ch.* **2012**, *92*, 1262-1275.
13. Trzonkowska, L.; Leśniewska, B.; Godlewska-Żyłkiewicz, B. *Crit. Rev. Anal. Chem.* **2016**, *46*, 305-322.
14. Novotnik, B.; Zuliani, T.; Ščančar, J.; Milačič, R. *J. Anal. At. Spectrom.* **2013**, *28*, 558-566.
15. Thor, M. Y.; Harnack, L.; King, D.; Jasthi B.; Pettit, J. *J. Food Comp. Anal.* **2011**, *24*, 1147-1152.
16. Ščančar, J.; Milačič, R. *J. Anal. At. Spectrom.* **2014**, *29*, 427-443.
17. Markiewicz, B.; Komorowicz, I.; Sajnóg, A.; Belter, M.; Baralkiewicz, D. *Talanta* **2015**, *132*, 814-828.
18. Kovachev, N.; Aguirre, M. A.; Hidalgo, M.; Smitchiev, K.; Stefanova, V.; Kmetov, V.; Canals, A. *Microchem. J.* **2014**, *117*, 27-33.
19. Barałkiewicz, D.; Pikosz, B.; Belter, M.; Marcinkowska, M. *Accredit. Qual. Assur.* **2013**, *18*, 391-401.
20. Chahl, V. K.; Singh, R.; Malik A. K.; Matysik, F. M.; Puri, J. K. *J. Chromatogr. Sci.* **2012**, *50*, 26-32.
21. Sun, J.; Ma, L.; Yang, Z.; Wang, L. *J. Sep. Sci.* **2014**, *37*, 1944-1950.
22. Jabłońska-Czapla, M.; Szopa, S.; Grygoyć, K.; Łyko, A.; Michalski, R. *Talanta* **2014**, *120*, 475-483.
23. Vaccina, V.; de la Calle, I.; Sèby, F. *Anal. Bioanal. Chem.* **2015**, *407*, 3831-3839.
24. Lopez-Garcia, I.; Briceño, M.; Vincente-Martinez, Y.; Hernandez-Córdoba, M. *Talanta* **2013**, *115*, 166-171.
25. Bahadir, Z.; Bulut, V. N.; Hidalgo, M.; Soylak, M.; Margui, E. *Spectrochim. Acta B* **2016**, *115*, 46-51.
26. Wen, S.; Wu, J.; Zhu, X. *J. Mol. Liq.* **2013**, *180*, 59-64.
27. Vidal, L.; Silva, S. G.; Canals, A.; Nóbrega, J. A. *Talanta* **2016**, *148*, 602-608.
28. Abadi, M. D. M.; Chamsaz, M.; Arbab-Zavar, M. H.; Shemirani, F. *Anal. Meth.* **2013**, *5*, 2971-2977.

29. Ezoddin, M.; Abdi, K.; Esmaeli, N. *Microchem. J.* **2016**, *120*, 200-204.
30. Meeravali, N. N.; Manjusha, R.; Kumar, S. J. *J. Anal. At. Spectrom.* **2014**, *29*, 2168-2175.
31. López-Garcia, I.; Vincente-Martinez, Y.; Hernández-Córdoba, M. *Talanta* **2015**, *132*, 23-28.
32. Pyrzynska, K. *TrAC-Trend. Anal. Chem.* **2013**, *43*, 100-108.
33. Płotka-Wasylka, J.; Szczepańska, N.; de la Guardia, M.; Namieśnik, J. *TrAC-Trend. Anal. Chem.* **2016**, *77*, 23-43.
34. Lopez-Lorente, A. I.; Simonet, B. M.; Valcárcel, M. *Anal. Bioanal. Chem.* **2011**, *399*, 43-54.
35. Diniz, K. M.; Tarley, C. R. T. *Microchem. J.* **2015**, *123*, 185-195.
36. Wu, Y. W.; Zhang, J.; Liu, J. F.; Chen, L.; Deng, Z. L.; Han, M. X.; Wei, X. S.; Yu, A. M.; Zhang, H. L. *Appl. Surf. Sci.* **2012**, *258*, 6772-6776.
37. Rezvani, M.; Asgharinezhad, A. A.; Ebrahimzadah, H.; Shekari, N. *Microchim. Acta* **2014**, *181*, 1887-1895.
38. Cui, C.; He, M.; Chen, B.; Hu, B. *Anal. Meth.* **2014**, *6*, 8577-8583.
39. Sereshi, H.; Farahani, M. V.; Baghdani, M. *Talanta* **2016**, *146*, 662-609.
40. Djerahov, L.; Vasileva, P.; Karadjova, I. *Microchem. J.* **2016**, *129*, 23-28.
41. Bahadir, Z.; Bulut, V. N.; Hidalgo, M.; Soylak, M.; Margui, E. *Spectrochim. Acta B* **2015**, *107*, 170-177.
42. Saçmacı, Ş.; Kartal, Ş.; Yılmaz, Y.; Saçmacı, M.; Soykan, C. *Chem. Eng. J.* **2012**, *181-182*, 746-753.
43. Şahan, S.; Saçmacı, Ş.; Kartal, Ş.; Saçmacı, M.; Şahin, U.; Ülgen, A. *Talanta* **2014**, *120*, 391-397.
44. Lopez Guerro, M. M.; Vereda Alonso, E.; Cano Pavón, J. M.; Siles Cordero, M. T.; Garcia de Torres, A. *J. Anal. At. Spectrom.* **2012**, *27*, 682-688.
45. Jia, X.; Gong, D.; Xu, B.; Chi, Q.; Zhang, X. *Talanta* **2016**, *147*, 155-161.
46. Corazza, M. Z.; Ribeiro, E. S.; Segatelli, M. G.; Tarley, C. R. T. *Microchem. J.* **2014**, *112*, 18-26.
47. Wang, Z.; Fang, D. M.; Li, Q.; Zhang, L. X.; Qian, R.; Zhu, Y.; Qu, H. Y.; Du, Y. P. *Anal. Chim. Acta* **2012**, *725*, 81-86.
48. Tarley, C. R. T.; Lima, G. F.; Nascimento, D. R.; Assis, A. R. S.; Ribeiro, E. S.; Diniz, K. M.; Bezerra, M. A.; Segatelli, M. G. *Talanta* **2012**, *100*, 71-78.
49. Ma, J.; Wang, Z.; Li, Q.; Gai, R.; Li, X. *J. Anal. At. Spectrom.* **2014**, *29*, 2315-2322.
50. Hsu, K. C.; Sun, C. C.; Ling, Y. C.; Jiang, J.; Huang, Y. L. *J. Anal. At. Spectrom.* **2013**, *28*, 1320-1326.
51. Chen, S.; Zhu, S.; He, Y.; Lu, D. *Food Chem.* **2014**, *150*, 254-258.
52. Abdolmohammad-Zadeh, H.; Sadeghi, G. H. *Talanta* **2012**, *94*, 201-208.
53. Alves, V. N.; Coelho, N. M. M. *Microchem. J.* **2012**, *109*, 16-22.
54. Peng, H.; Zhang, N.; He, M.; Chen, B.; Hu, B. *Talanta* **2015**, *131*, 266-272.
55. Leśniewska, B.; Trzonkowska, L.; Zambrzycka, E.; Godlewska-Żyłkiewicz, B. *Anal. Meth.* **2015**, *7*, 1517-1525.
56. Socas-Rodriquez, B.; Herrera-Herrera, A. V.; Asensio-Ramos, J. M.; Hernández-Borgez, J. *J. Chromatogr. A* **2014**, *1357*, 110-146.
57. EFSA Panel on Contaminants in the Food Chain. *EFSA Journal* **2014**, *12*, 3595.
58. Vieira, E.; Soares, E.; Kozior, M.; Krejpcio, Z.; Ferreira, I. M. P. L; Bastos, M. L. *J. Agric. Food. Chem.* **2014**, *62*, 9195-9200.
59. Lameiras, J.; Soares, M. E.; Bastos, M. L.; Ferreira, M. *Analyst* **1998**, *123*, 2091-2095.
60. Soares, M. E.; Bastos, M. L.; Ferreira, M. *J. AOAC Int.* **2000**, *83*, 220-223.
61. Panichev, N.; Mandiwana, K.; Kataeva, M.; Siebert, S. *Spectrochim. Acta B* **2005**, *60*, 699-703.
62. Elci, L.; Divriki, U.; Akdogan, A.; Hol, A.; Cetin, A.; Soylak, M. *J. Hazard. Mater.* **2010**, *173*, 778-782.
63. Mandivana, K. L,; Panichev, N.; Panicheva, S. *Food Chem.* **2011**, *129*, 1839-1843.

64. California Department of Public Health. *First Drinking Water Standard for Hexavalent Chromium Now Final*; CDPH: Sacramento, CA, USA, 2014.

65. Sharangi, A. B. *Food Res. Int.* **2009**, *42*, 529-535.

66. Hosseini, M. S.; Belador, F. *J. Hazard. Mater.* **2009**, *165*, 1062-1067.

67. Novotnik, B.; Zuliani, T.; Ščančar, J.; Milačič, R. *J. Trace Elem. Med. Biol.* **2015**, *31*, 98-106.

68. Lin, Y. A.; Jiang, S. J.; Sahayam, A. C.; Huang, Y. L. *Microchem. J.* **2016**, *128*, 274-278.

69. Ambushe, A. A.; McCrindle, R. I.; McCrindle, C. M. E. *J. Anal. At. Spectrom.* **2009**, *24*, 502-507.

70. Soares, M. E.; Vieira, E.; de Lourdes Bastos, M. *J. Agric. Food Chem.* **2010**, *58*, 1366-1370.